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APPLICATION NUMBER: 020807

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

AUG 20 1997

Clinical Pharmacology & Biopharmaceutics Review

NDA 20-807 Submission Date: 01-02-97, 03-27-97,
Refludan® Sterile Powder for Injection or Infusion
r-Hirudin 50 mg/vial

Sponsor: ClinTrials Research Inc.
Research Triangle Park, NC 27709

Priority: 1P
Reviewer: Rajendra S. Pradhan, Ph. D.

Type of Submission: NME

Synopsis:

The sponsor has submitted the application (NDA) 20-807, Refludan® Sterile Powder for Injection or Infusion, indicated as an antithrombotic agent only in patients who have heparin associated thrombocytopenia (HAT).

Refludan® (lepirudin) is a highly specific direct inhibitor of thrombin. Lepirudin is a recombinant hirudin derived from yeast cells. The activity of lepirudin is measured by a chromogenic assay. One anti-thrombin unit (ATU) is the amount of hirudin that neutralizes one unit of WHO preparation 89/588 of thrombin. Its mode of action is independent of antithrombin III. Platelet factor 4 does not inhibit lepirudin. Lepirudin and thrombin interacts in 1:1 proportion. Twenty-six clinical pharmacology studies were submitted by the sponsor in the section 6 of NDA 20-807, including the pivotal safety and efficacy clinical trial. The sponsor has also conducted a comprehensive population pharmacokinetic analysis to identify the pharmacokinetic parameters and effects of different demographic covariates and coadministration of other drugs on these PK parameters.

It is expected that metabolism of lepirudin proceeds by release of amino acids via catabolic hydrolysis of the parent drug. About 48.3% of administered dose was excreted in urine, which consisted of unchanged drug (~ 35%) and other fragments of the parent peptide chain. All the urinary fragments of parent drug (amino acid chain length > 60) retain anti-thrombotic activity. After an IV administration of lepirudin to healthy volunteers, Thrombin Hirudin Complex (THC)-levels only constituted less than 2% of lepirudin concentrations when expressed in hirudin equivalents. The sponsor is requesting approval for only IV (bolus and infusion) route administration of r-hirudin, however, when the drug was administered by a subcutaneous (SC) route in normals, absolute bioavailability estimates were 85% using urine data. The drug post SC administration show a slower rate of absorption (SC, Tmax = 2 to 2.5 hr) and a lower systemic clearance resulting in longer terminal half-life ($t_{1/2}$). Clearance

estimates for SC route is probably confounded by the non-specific assay used to estimate plasma r-hirudin levels. The dose linearity of clinically relevant doses was established in a three-way cross-over study involving 18 healthy subjects (study A3). In this study, total clearance and the renal clearance in females were on average two-third (2/3) that of males. There were no gender differences in aPTT response to doses In a parallel design IV infusion study in normals, a trend to linear pharmacokinetics was observed. Slightly higher AUCs were noted along with an increase of terminal half-life for elderly subjects, due to decrease in total and renal clearance. There was a linear relationship between creatinine clearance and drug clearance. The concentration effect relationship (plasma concentration vs aPTT) was similar in healthy subjects and in patients with impaired renal function. A dose reduction, directly proportional to reduction in CrCl is recommended based on data observed in study A 14 (which complements the results of population pharmacokinetic covariate analysis). In a clinical safety-efficacy study with Heparin Induced Thrombocythemia type II (HAT-II), patients with isolated thrombocytopenia who did not suffer from thromboembolic complications showed 30% lower clearance for drug than patients with thromboembolic complications at baseline, not undergoing thrombolysis.

In a population PK analysis of 644 patients from nine different studies (with 5669 measurable concentrations), r-hirudin's mean systemic clearance estimate for HAT-II patients was 6.86 L/hr (intersubject variability, %CV = 46.8). Relationship between aPTT ratio and plasma r-hirudin concentration was defined by a spline function with an intercept and slope parameter specific for the patient population studied. For patients with adjunctive thrombolysis and HAT type II patients, the variability of aPTT ratios was high, compared to healthy volunteers and other patients.

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The sponsor is proposing to market the same formulation that was used in pivotal safety-efficacy trial.

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Recommendation:

The Human Pharmacokinetics and Biopharmaceutics portion of NDA 20-807 is approved. Please forward the text under Comments to the sponsor as appropriate.

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/S/ 8-20-97

Rajendra S. Pradhan, Ph.D.
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FT initialed by Lydia Kaus, Ph.D. /S/ 8/20/97

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cc: NDA 20-807, HFD-180, HFD-870 (MChen, Kaus, Pradhan), HFD-850 (Lesko), HFD-340 (Viswanathan), HFD-850 (Drug, Reviewer), Central Document Room (Barbara Murphy)

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Background: Refludan® (lepirudin) is a highly specific direct inhibitor of thrombin. Lepirudin is a recombinant hirudin derived from yeast cells. Natural hirudin is produced in trace amounts as a family of highly homologous isopolypeptides by the leech Hirudo medicinalis. The activity of lepirudin is measured by a chromogenic assay. One anti-thrombin unit (ATU) is the amount of hirudin that neutralizes one unit of WHO preparation 89/588 of thrombin. Its mode of action is independent of antithrombin III. Platelet factor 4 does not inhibit lepirudin. Lepirudin and thrombin interacts in 1:1 proportion. Lepirudin is indicated for anticoagulation in adult patients with heparin-associated thrombocytopenia (HAT) type II and thromboembolic disease[1]. The proposed to-be-marketed formulation is a sterile white freeze-dried powder for injection or infusion and is freely soluble in water for injection or isotonic saline.

Currently, unfractionated heparin (UFH) is used for a wide range of thrombotic disorders, including acute coronary syndromes. It is the only anticoagulent that can be used for cardiovascular surgical procedure at present. Its antithrombotic action depends on the presence of endogenous coagulation inhibitor factors, ATIII and heparin cofactorII. There are several limitations to UFH for their use as a antithrombotic agent. It is a heterogenous mixture and therefore all molecules do not have the same antithrombin potency (only one third of chains constituting the heparin molecules possess a pentasaccharide sequence, which is required to bind ATIII). Heparin can be inactivated by heparinase and platelet factor IV, both of which are released by activated platelets. UFH binds to vitronectin, fibronectin and other plasma proteins thus limiting the amount of antithrombotic heparin available. Most important, however, is the inability of the heparin-AT III complex to inactivate thrombin already bound to clots. This is a major drawback to heparin use, because clot bound thrombin can act as an ongoing source of thrombogenesis at sites of pathologic thrombus formation. Because of the substantial limitations of available therapy, there is a need for a better antithrombotic agent.

Direct thrombin inhibitors such as r-hirudin enables one to control the thrombogenic effects of thrombin by interacting directly with it, thereby preventing its interaction with its substrates in AT III and heparin cofactor II independent fashion. The amino terminus of hirudin interacts with the apolar binding site of thrombin, whereas the carboxy terminus, which is highly acidic, interacts with the binding exosite for fibrinogen recognition. Because of this dual interaction r-hirudin is a highly specific inhibitor of thrombin.

Although hirudin is an effective anticoagulant, it is short lived (pharmacokinetically) and may exhibit immunogenic behavior. Also, at present an antidote to hirudin is not present to stop unwanted abnormal bleeding. In NDA 20-807, the sponsor is therefore requesting approval of r-hirudin as a antithrombotic only in patients who have heparin associated thrombocytopenia (HAT).

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[1] Brief explanation is provided under "CLINICAL TRIAL DATA" in proposed labelling text

Twenty-six clinical pharmacology studies were submitted by the sponsor in the section 6 of NDA 20-807, including the pivotal safety and efficacy clinical trial. Out of twenty-six studies, ten studies were conducted in healthy subjects (total = 124 subjects), seven were PK-PD studies in special population (562 subjects including females, elderly, renally impaired, thrombolysed AMI patients, HAT type II), eight were other less important/pilot studies (103 subjects including Japanese studies and pilot studies) and a drug formulation comparison study (18 subjects). The sponsor has also conducted a comprehensive population pharmacokinetic analysis to identify the pharmacokinetic parameters and effects of different demographic covariates and coadministration of other drugs on these PK parameters. The sponsor included data from nine important studies including the pivotal safety/efficacy study in this population PK analysis (N = 644). Twelve studies were considered to be primary and rest to be supportive.

Summary (Chemistry, Metabolism, Pharmacokinetics and Pharmacodynamics):

r-Hirudin, shown in the following figure is a direct thrombin inhibitor.

```
LeuThrTyrThrAspCysThrGluSerGlyGlnAsnLeuCysLeu
1             5                 10              15

CysGluGlySerAsnValCysGlyGlnGlyAsnLysCysLleLeu
16            20                25              30

GlySerAspGlyGluLysAsnGlnCysValThrGlyGluGlyThr
31            35                40              45

ProLysProGlnSerHisAsnAspGlyAspPheGluGluIlePro
46            50                55              60

GluGluTyrLeuGln
61            65
```


Disulfide Bonds: Cys6 - Cys6, Cys16 - Cys28 and Cys22-Cys39.

Mass Balance/Metabolism:

It is expected that metabolism of lepirudin proceeds by release of amino acids via catabolic hydrolysis of the parent drug. However, the sponsor has not characterized the catabolic process in man in greater detail.

Total urinary excretion attributed to lepirudin and four metabolites was 48.3%

of the administered dose. Metabolites arise from C-terminal cleavage of amino acids and distributed as follows:

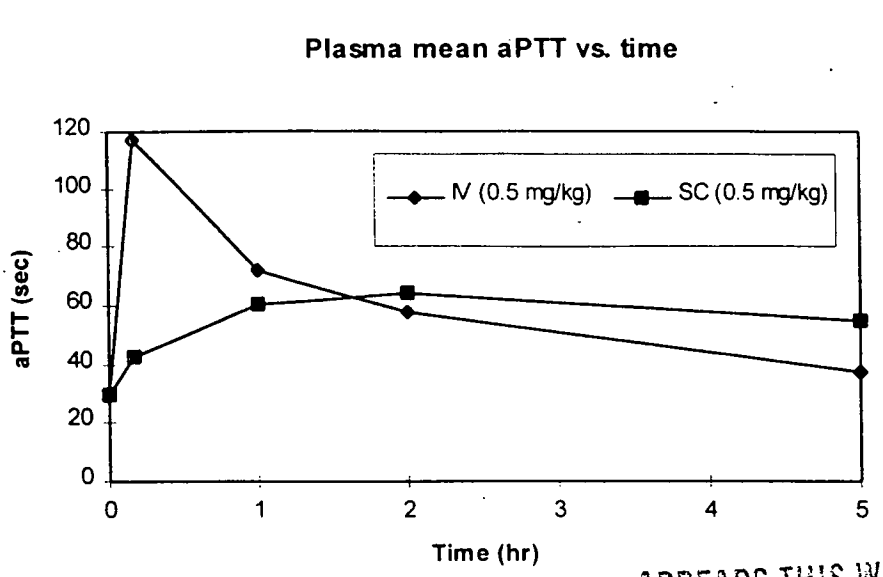
M0 (parent drug, 1-65)	=	35%
M1 (1-64)	=	2.5%
M2 (1-63)	=	5.4%
M3 (1-62)	=	3.9%
M4 (1-61)	=	1.6%

The metabolites retain anti-thrombotic activity and hence are detectable e.g.

After an IV administration of lepirudin to healthy volunteers, Thrombin Hirudin Complex (THC)-levels only constituted less than 2% of lepirudin concentrations when expressed in hirudin equivalents. The metabolic fate of circulating THC derived from lepirudin is unknown. The elimination half-life of THC is about 3 hours (as oppose to 1.3 hours for lepirudin). If any, a very small contribution on pharmacodynamics of THC with its longer half-life is conceivable; neither aPTT measurements , however, could reflect the existence of such a slow second elimination half-life.

Absorption:

In this application (NDA 20-807) the sponsor is seeking approval only for IV bolus and infusion routes of administration. The sponsor has conducted 9 secondary pharmacokinetic studies in which lepirudin was administered subcutaneously (SC). These studies were not designed to estimate absolute bioavailability of SC route (IV arm absent). The sponsor carried out a formal absolute-bioavailability study with a 2-way cross-over design in 12 healthy subject, this study shows absolute bioavailability of SC route to be 111% based on plasma data and 85% based on urine data. The drug post SC administration, show a slower rate of absorption compared to the IV route (SC, Tmax = 2 to 2.5 hr) and systemic clearance appears to be lower for SC route resulting in longer terminal half-life ($t_{1/2}$). It is suspected that SC metabolic profile may be different compared to IV route; the assay is unable to differentiate between metabolites (different fragments of r-Hirudin) so an inflated absolute bio-estimate is generated. For this absolute bioavailability study the sponsor used plasma concentrations of r-hirudin.



Rate of absorption of r-hirudin from SC site is higher for females than for males.

Pharmacokinetics/Dose Proportionality:
The dose linearity of clinically relevant doses of was assessed in a three-way cross-over study involving 18 healthy subjects (study A3). Production scale material of lepirudin was used in this study

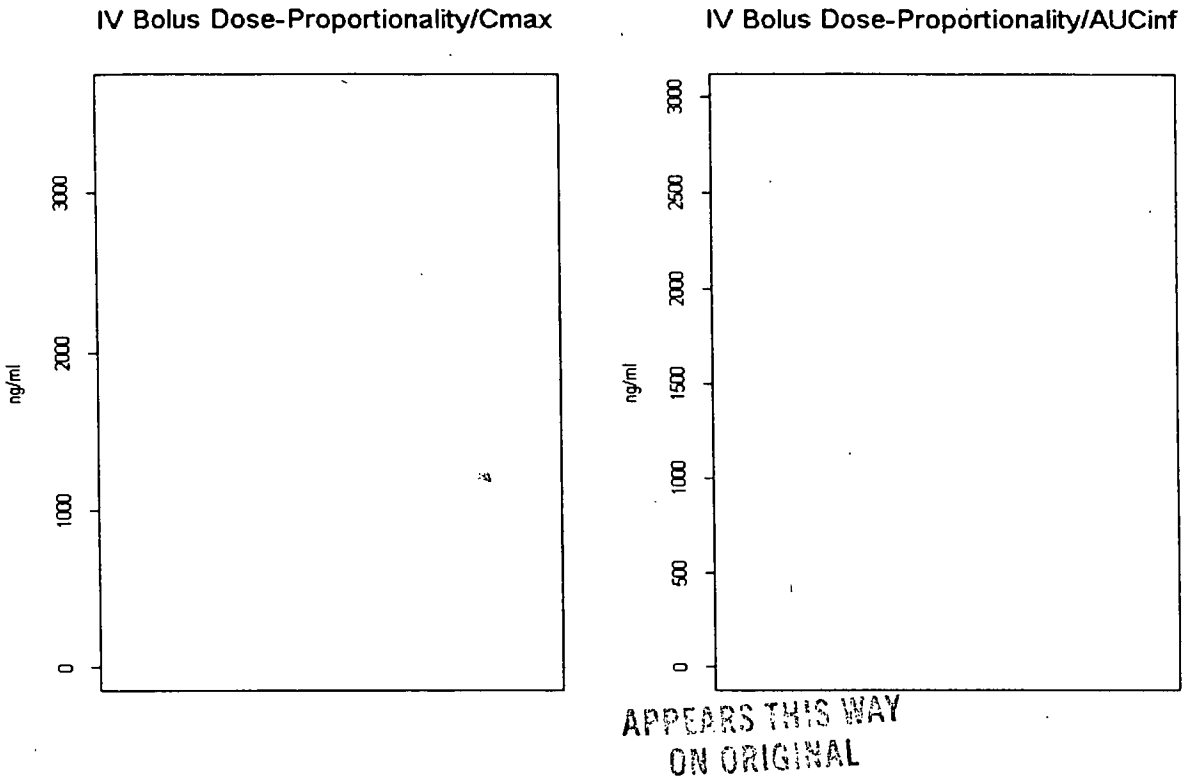
Mean (CV%) and range

		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Plasma data:				
C_{max} (ng/ml)	Males	793(14.2)	1500(11.0)	2927(8.58)
	Females	835(10.4)	1433(7.56)	2922(10.8)
AUC_{last} (ng.h/ml)	Males	591(19.0)	1165(15.1)	2328(9.25)
	Females	708(13.5)	1265(7.73)	2511(11.0)
$AUC_{0-\infty}$ (ng.h/ml)	Males	629(18.8)	1220(14.9)	2407(9.50)
	Females	757(16.2)	1318(7.77)	2594(11.5)
$t_{1/2;\alpha}$ (h)	Males	0.16(35.8)	0.19(43.8)	0.26(32.2)
	Females	0.26(34.0)	0.21(38.0)	0.22(48.2)

		0.19-0.40	0.11-0.37	0.13-0.40
$t_{1/2,b}$ (h)	Males	0.99(22.2)	1.22(28.9)	1.35(18.6)
	Females	1.43(38.1)	1.33(17.1)	1.32(14.9)
CL_{tot} (ml/min)	Males	203(18.2)	208(15.7)	207(11.8)
	Females	138(20.9)	154(13.0)	157(12.0)
MT_{vss} (h)	Males	1.28(20.6)	1.46(18.7)	1.48(11.7)
	Females	1.64(30.0)	1.64(15.6)	1.56(8.02)
V_{ss} (l)	Males	15.3(15.6)	17.9(16.5)	18.3(14.3)
	Females	12.9(13.6)	15.1(14.3)	14.7(11.4)
Urine data:		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Ae(0-48h) (mg)	Males	2.99(23.8)	6.07(27.8)	14.5(10.0)
	Females	2.20(27.8)	4.62(24.2)	10.2(13.7)
Ae(0-48h) (% of dose)	Males	40.5(24.6)	41.2(30.4)	49.2(15.0)
	Females	35.0(25.1)	37.3(19.1)	42.2(11.1)
CL_{ren} (ml/min)	Males	82.0(29.3)	85.8(34.6)	100(12.4)
	Females	51.9(31.7)	59.0(23.2)	66.5(17.7)

The PK parameters Cmax, AUC and Ae showed a fairly linear increase with dose. The same was true for the course of plasma levels as a whole. Hence, systemic clearance and Vss were fairly equal across the three doses applied. A slight increase of renal clearance from lower dose to higher dose was observed; this resulted in higher percentage of dose excreted in urine for the highest dose. This difference appears to be clinically irrelevant. The total clearance and the renal clearance in females were on average two-third (2/3) that of males.

The following figure illustrate the dose linearity of Cmax and $AUC_{0-\infty}$ parameters for 0.1, 0.2 and 0.4 mg/kg doses.



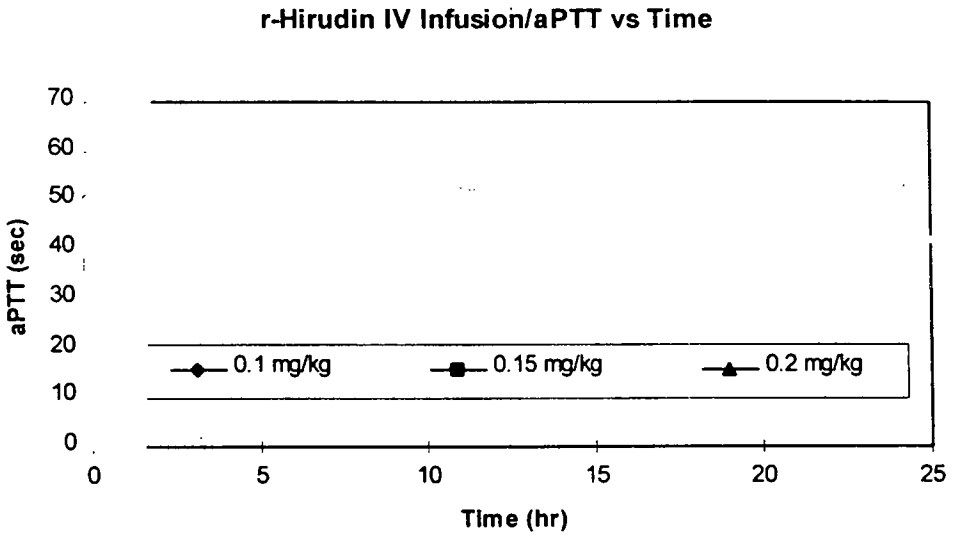
Pharmacokinetics and pharmacodynamics of single dose 0.1, 0.15 and 0.2 mg/kg r-hirudin, given as IV infusion over six hours was studied in healthy males. The following table summarizes the means and standard deviations of the pharmacokinetic parameters of hirudin

Parameters (Mean , SD)	0.1 mg/kg	0.15 mg/kg	0.2 mg/kg
Cmax (ng/ml)	111 (17.6)	203 (19.9)	246 (25.8)
AUC_{0-6h} (ng.h/ml)	469 (68.4)	850 (76.1)	1109 (123)
AUC_{6-11h} (ng.h/ml)	142 (32.0)	252 (53.8)	277 (49.0)
AUC (ng.h/ml)	612 (122)	1184 (152)	1446 (123)
t1/2 a (h)	0.07 (0.04)	0.16 (0.10)	0.12 (0.09)
t1/2 b (h)	1.10 (0.42)	1.98 (0.86)	1.36 (0.40)
Cltot (ml/min)	208 (43.7)	160 (32.6)	189 (14.7)
Vss (l)	17.1 (4.71)	17.0 (2.88)	16.0 (3.62)

Pharmacokinetic parameters in urine:

Parameters (Mean, SD)	0.1 mg/kg	0.15 mg/kg	0.2 mg/kg
Ae (0-12 h) mcg	2517 (608)	4380 (723)	5557 (2102)
Ae (0-12 h) (%dose)	34.1 (8.26)	39.4 (7.33)	33.4 (10.9)
Clren(0-12 h) ml/min	69.0 (18.6)	66.6 (14.8)	65.9 (22.6)

This was a parallel study design and therefore it was difficult to make PK linearity conclusions over the studied dose range. The following figure shows the aPTT-time profile for three different IV infusion doses.

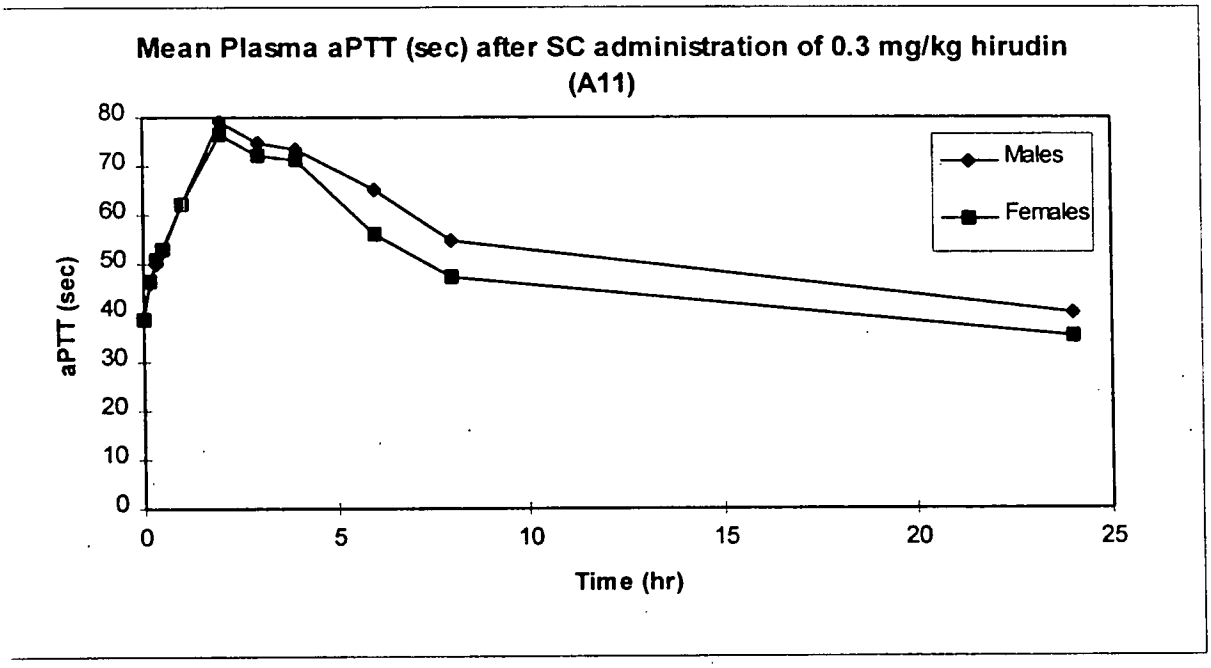


Pharmacokinetics Pharmacodynamics in Special Population/Patients

Females:

After IV or SC injection of lepirudin into healthy male or female volunteers, clearance of the drug was Urinary excretion of unchanged drug and renal clearance were lower in female subjects (which might be due to incomplete urine collection in the female volunteers or to slightly lower glomerular filtration rate or to increased intrarenal metabolism).

After SC administration, terminal half-life was significantly reduced by about 20% in women. Rate of absorption from the site of injection was more rapid in women. There was no relevant sex difference in pharmacodynamic response.



Overall, it appears that there are moderate differences in PK between males and females, however, this does not translate into similar differences in PD (aPTT).

Elderly:

The PK and PD of lepirudin in the elderly have been investigated after IV (bolus) administration to five healthy males and five healthy females

in study A 12. Slightly higher AUCs were noted along with an increase of terminal half-life for elderly subjects, due to decrease in renal clearance. The following table compares PK parameters of r-hirudin after an IV administration of 0.1 mg/kg r-hirudin to elderly subjects (A12) to PK parameters seen in healthy young subjects at the same dose level (A3).

	Elderly Subjects	Young Subjects
Study N Age range (yr)	A12 10 Mean (SD)	A3 18 Mean (SD)
Cmax (ng/ml)*	668 (144)	814 (99.6)
$AUC_{0-\infty}$ (ng.h/ml)	778 (194)	693 (134)
terminal t1/2 (hr)	1.7 (0.5)	1.2 (0.5)
Vss	16.0 (2.6)	14.1 (2.4)
CL* (ml/min)	182.0 (33.8)	170 (46.4)
CLr (ml/min)	61.8 (22.1)	68.8 (25.6)
Ae_{0-48} (% of dose)	40.8 (11.0)	38.1 (9.6)

*: CL0-5hr for elderly subjects

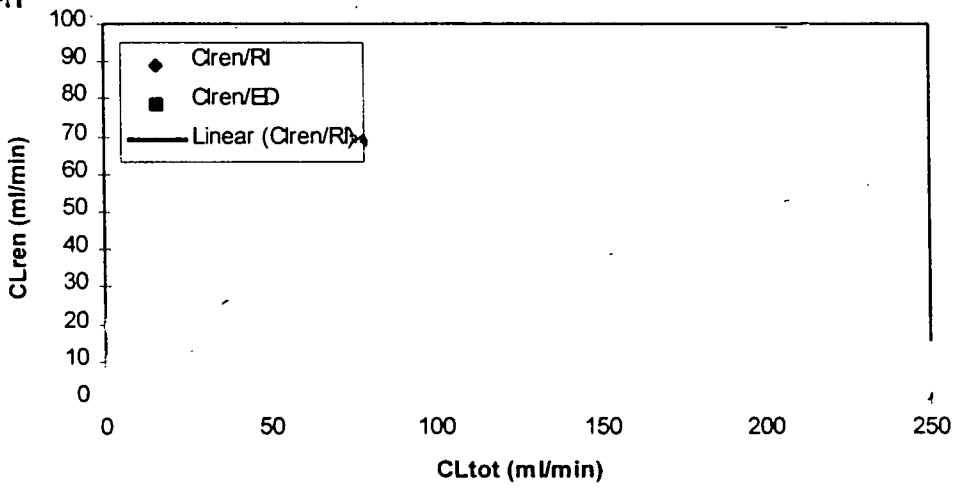
The following table compare aPTT values (sec) after an IV administration of 0.1 mg/kg r-hirudin to elderly subjects (A12) to aPTT values seen in healthy young subjects at the same dose level (A3).

	Elderly Subjects	Young Subjects
Study N Age range (yr) Time	A12 10 Mean (SD)	A3 18 Mean (SD)
0	33.1 (4.0)	30.6 (2.2)
0.167	76.4 (10.5)	77.1 (8.5)
0.333	65.9 (8.1)	-
0.5	60.8 (6.9)	-
1	55.3 (6.3)	51.6 (6.4)
2	49.8 (6.8)	42.3 (4.7)
3	44.8 (5.0)	-

4	43.6 (4.7)	-
5	-	35.0 (4.2)
6	39.1 (4.6)	-

An analysis of the correlation between the renal and total clearance values for lepirudin show that the same relationship applies for the elderly population and for patients with kidney disease.

CLren vs CLtot comparison between elderly and renally impaired



It appears that no specific dosage adjustment is necessary in elderly patients, apart from adjustment for creatinine clearance, if necessary.

Renal Impairment:

Study A14 assessed the pharmacokinetics and pharmacodynamics of lepirudin in 16 patients mean 47.8 yr) with kidney disease at 0.05 mg/kg dose (IV infusion given over 60 min). There was a linear relationship between creatinine clearance and drug clearance. The following table shows the mean values and ranges of the pharmacokinetic variables.

Plasma:

Variable	Group I (n = 3)	Group II (n = 5)	Group III (n = 5)	Group IV (n = 3)
CrCl (ml/min)	80-50	49.9-20	19.9-10	< 10
C_{max} [1] (ng/ml)	260	315	404	340
AUCinf (ng.h/ml)	433	1028	3214	12737
$t_{1/2;b}$ (h)	1.35*	2.72	6.72	9.69*
CL_{tot} (ml/min)	168	95.9	28.8	4.74
V_{ss} (l)	11.5	16.3	23.6	22.4
1) at the end of infusion		*		n = 2

Urine:

The mean values and ranges of the total urinary excretion calculated up to the last measuring point per group (Ae) and average renal clearance (Clren) of r-hirudin were as follows

Variable	Group I (0 - 48h) (n = 3)	Group II (0 - 96h) (n = 5)	Group III (0 - 120h) (n = 5)	Group IV (0 - 120h) (n = 3)
Ae (% of dose)	29.2	35.2	50.6	50.6
CL_{ren} (ml/min)	45.7	32.5	15.5	2.77

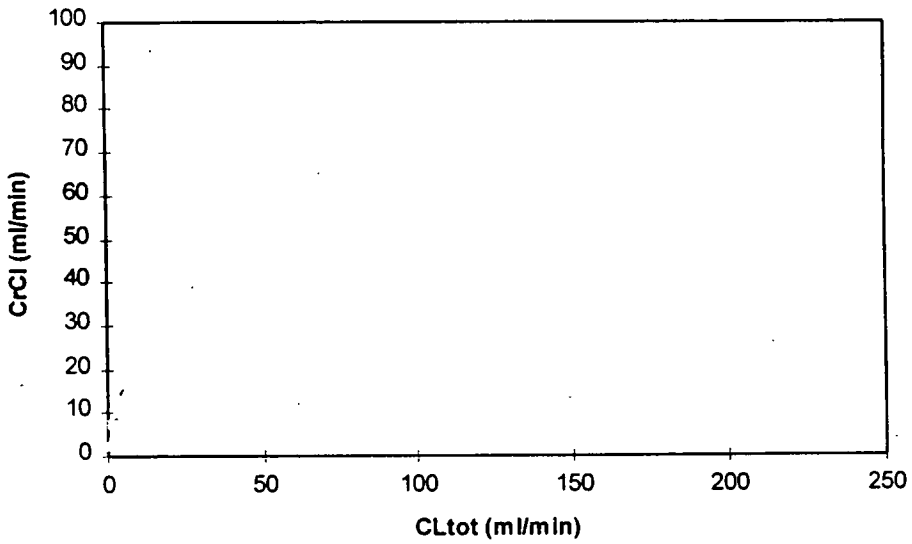
For the most extreme situation of hemodialysis patients, elimination half-lives of about two days were observed. The concentration effect relationship (plasma concentration vs aPTT) was

similar in healthy subjects and in patients with impaired renal function.

The mean values and ranges of aPTT (seconds) at a few selected sampling times were

Sampling time (h)	Group I (n = 3)	Group II (n = 5)	Group III (n = 5)	Group IV (n = 3)
0	32.0**	30.6	37.6	36.0
1	54.7	57.2	81.2	96.3
5	32.3	38.5****	47.6	57.3
24	30.0	30.8****	65.6	48.0
72	23.0*	31.8****	33.0***	36.0**
*	n = 1		***	n = 3
**	n = 2		****	n = 4

CLtot vs CrCl in Renally Impaired Patients



A dose reduction, directly proportional to reduction in CrCl is recommended based on data observed in this study.

Acute Myocardial Infarction (thrombolysis):

In a clinical safety and efficacy trial (B1), 143 patients 115 males, 28 females) suffering from acute myocardial infarction (AMI) received an IV bolus of r-hirudin followed by a constant rate infusion over 48 hours

These patients were undergoing thrombolytic therapy with tissue plasminogen activator (t-PA). Serial blood samples were collected up to 72 hours after medication for the determination of r-hirudin plasma concentration. Additionally, in a clinical safety and efficacy trial (B2), 272 AMI patients (222 males, 50 females)received an IV bolus or r-hirudin followed by a constant rate infusion up to 72 hours

These patients were undergoing thrombolytic therapy with streptokinase. Serial blood samples were collected up to 84 hours after medication for the determination of r-hirudin plasma concentration.

The steady state concentrations increased with the rate of infusion, but were comparable for t-PA and streptokinase coadministration. Systemic clearance was independent of dose.

	Study (B1)		Study (B2)	
	N	Clearance (mean, sd)	N	Clearance (mean, sd)
Males	111	157.5 (56.3)	145	136.8 (59.3)
Females	26	129.2 (49.2)	28	131.4 (12.4)
Age < 60	80	165.6 (60.3)	92	145.3 (56)
60-70	50	132.5 (44.1)	63	117.1 (33.2)
>70	7	138.1 (37.8)	18	153.3 (177.2)
Scr (mg/dl)				
<1	66	165.8 (55.3)	132	138.9 (73.5)
1-1.3	48	152.1 (57.2)	37	129.7 (74.7)
>1.3	21	111.2 (35.6)	3	91.7 (49.8)

aPTT values also increased in a dose dependent manner; aPTT values were higher for B2 (streptokinase) than for B1 (t-PA), especially during the first 24 hours after thrombolytic treatment.

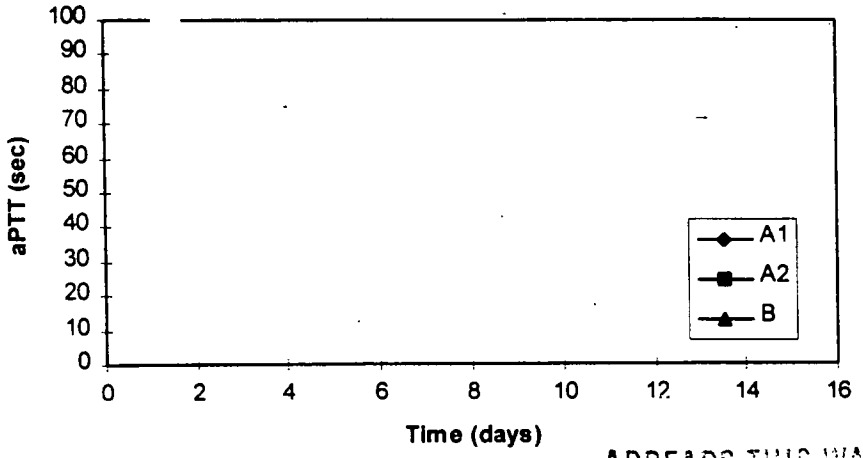
Heparin associated thrombocytopenia (HAT) type II:
The following table summarizes the pharmacokinetics of r-hirudin in HAT type II patients.

A1: 0.4 mg/kg bolus followed by 0.15 mg/kg infusion for 2-10 days (patients with thromboembolic complications at baseline, not undergoing thrombolysis)
A2: 0.2 mg/kg bolus followed by 0.1 mg/kg infusion for 2-10 days (patients with thromboembolic complications at baseline, undergoing thrombolysis)
B: 0.1 mg/kg infusion for 2-10 days (patients with isolated thrombocytopenia who did not suffer from thromboembolic complications)

	N	Clearance (mean, SD)
Dose Group		
A1	50	132.4 (91.6)
A2	5	114.6 (55.3)
B	18	93.7 (43.7)
Sex		
Males	26	139.5 (51)
Females	47	111.7 (93.1)
Age (yr)		
<60	36	129.4 (51.9)
60-70	21	111.2 (39.8)
>70	16	118.0 (151.8)
Serum Creatinine (mg/dl)		
<1.1	49	134.4 (92.6)
1.1-1.5	6	109.6 (31.3)
>1.5	7	68.2 (48.9)

In spite of a lower infusion rate in group B, steady state concentration values were comparable to group A1, reflecting a nearly 30% lower hirudin clearance for group B. The course of aPTT values showed slightly higher values for regimen A1, as compared to B.

Plasma aPTT values after administration of varying IV infusions of r-Hirudin to patients with HAT type II



PK-PD Changes in the presence of anti-hirudin antibodies (animal Study)

An important aspect of prolonged or repeated treatment with lepirudin is the potential influence of antibodies formed against the drug (proposed indication is only for 2 to 10 days of administration). In the HAT-type II treatment study, IgG antibody formation was observed but it did not result in allergic reactions. In few cases, IV infusion rates had to be reduced, in order to maintain the desired aPTT levels recommended in the study protocol.

This issue was specifically studied in cynomolgues monkeys by conducting a 13 week repeated administration toxicity study. Three groups of animals (total =28) were treated with IV bolus doses of 1, 10 or 30 mg/kg per day. PK was recorded on day 1 and day 89 where as PD data were recorded on day 1 and day 85. The animals were classified according to whether antibodies had formed by day 89.

PK assessments (Day 89)	Antibody by day 89= No		Antibody by day 89= Yes	
	Day 1	Day 89	Day 1	Day 89
Number of **Animals**	13	13	14	9
Elimination **half-life** (hr)	1.86	1.28	1.89	3.39
Systemic clearance (ml/min/kg)	5.16	5.24	5.15	2.76
Vd at steady state (L/kg)	0.40	0.28	0.40	0.5

PD assessments (Day 85)/aPTT trough (sec)	Antibody by day 89= No	Antibody by day 89= Yes
dose = 1 mg/kg/day	21.7	25.8
dose = 10 mg/kg/day	22.6	31.6
dose = 30 mg/kg/day	21.7	33.9

Thus, it appears that elimination of r-hirudin slows down by a factor 2-3 in the presence of antibodies.

Population Pharmacokinetic Analysis:

The following studies were included in a population kinetic analysis, in order to test covariate-effects on clearance.

Study	# of subjects
A3 (dose linearity)	18
A4 (young healthy)	15
A12 (elderly)	10
A14 (renal impairment)	16
B1 (AMI/ rtPA lysis)	143
B2 (AMI/ SK lysis)	202
B4 (unstable angina)	40
B6 (deep venous thrombosis)	118
B7 (HAT-II)	82
Total Subjects	644

The overall population had following characteristics.

Patient/Subject Demographics Summary (N=644)	
Demography	Mean (SD)
Age (years)	56.83 (14.37)
Weight (kg)	77.58 (12.53)
Creatinine clearance (ml/min)	89.43 (33.86), N=634*
Gender (%)	70.3% male, 29.7% female
Race (%)	97.5% caucasians, 2.5% others
Aspirin concomitant treatment	60.4% yes, 39.6% no
tPA concomitant treatment	23.0% yes, 77.0% no
Streptokinase concomitant treatment	32.3% yes, 67.7% no
Coumarin concomitant treatment	12.0% yes, 88.0% no (Incorrect**)

Heparin concomitant treatment	16.8% yes, 83.2% no
*: There are 5 patients from study 7MN-201 with incorrect serum creatinine concentration units which led to creatinine clearance values of > 300 ml/min. These values are not included in this summary. **:In reviewer's calculation this number was much less than 12.0%.	

Plasma r-hirudin concentration-time data were analyzed by nonlinear mixed-effects modeling (NONMEM, Version IV) to develop a population pharmacokinetic model using the first-order approximation method. A two-compartment model with rapid bolus or constant rate IV infusion was chosen as a base model. Potential covariates identified in the screening process were added to the expression of an individual PK parameter incrementally and tested to determine if they were indeed significant. The following table shows the result of the covariate analysis.

Covariates confirmed as significant by NONMEM	
Systemic Clearance (CL)	AGE, CRCL, GENDER
Volume of Distribution of the Central Compartment	GENDER, ANYH (any heparin), ANYA (any aspirin)
Volume of Distribution of the Peripheral Compartment (V2)	DOSE, ANYS (any streptokinase)
Intra-subject Variability	

The population pharmacokinetic model is summarized as follows:

where GENDER: 1= male, 2 = female

POSTHOC Estimates:
Based on the full model described above, PK parameters were calculated for individual subjects and patients. Theses results [mean (%CV)] are summarized in the following table for each study.

thombolyzed patients, which suggests an interesting drug-interaction of the thrombolytic agents (rt-PA and streptokinase).

Population	Samples	$a_0 \pm SE$	$a_1 \pm SE$
Healthy Subjects	395	-2.328 ± 0.039	0.879 ± 0.014
Thrombolysis (rt-PA)	810	-0.824 ± 0.114	0.384 ± 0.038
Thrombolysis (SK)	900	-1.385 ± 0.101	0.586 ± 0.035
HAT type II	580	-1.918 ± 0.217	0.668 ± 0.069
Other	1106	-2.556 ± 0.141	0.824 ± 0.045

The following table shows model predicted aPTT-ratios for various populations at different concentrations.

Plasma Conc. (μg/ml)	Healthy Subjects	Thrombolysis		HAT type II	Other
		rt-PA	SK		
0.25	1.74	2.15	2.03	1.66	1.47
0.5	2.06	2.36	2.33	1.86	1.65
1.0	2.57	2.62	2.74	2.13	1.89
1.5	3.01	2.80	3.05	2.34	2.09
2.0	3.42	2.95	3.33	2.52	2.27

The aPTT ratios are defined as ratio of aPTT at time "t" to baseline aPTT.

The PK-PD model proposed by the sponsor is purely empiric. It does not take into account the mechanism of anticoagulant action of r-hirudin. A suitable hyperbolic function might have been a better choice in describing r-hirudin concentration and aPTT (or aPTT ratio) relationship.

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Comments:

2. In study A4 (IV infusion), assessment of pharmacokinetic linearity was limited by the parallel study design.

3. In the pivotal clinical trial in HAT-II patients, group B (patients with isolated thrombocytopenia without thromboembolic complications) showed about 30% lower r-Hirudin clearance than group A1 or A2 (patients with thromboembolic complications). The sponsor is requested to address/explain this observation.

4. Lepirudin when administered subcutaneously, showed an absolute bioavailability of $>100\%$.

5.

6. In the population PK analysis the sponsor did not attempt any model validation. A good strategy would The model developed based on the rest of the data could then be tested for its prediction performance on the set aside data. Other practical model approaches could also be useful and effective[2].

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[2] Ette, E. Stability and performance of a population pharmacokinetic model, J. Clin. Pharmacol 1997; 37: 486-495

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Appendix I

Assay Methods

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Pharmacokinetics Including Dose Linearity, Pharmacodynamics and Tolerability of 0.1, 0.2 and 0.4 mg/kg r-Hirudin Administered Intravenously (bolus) in Healthy Volunteers

Study #: A3

Study Objectives:

To investigate the pharmacokinetics including dose linearity, pharmacodynamics and tolerability of intravenously (bolus) administered r-hirudin in healthy volunteers.

Study Design:

Single-blind, randomized, 3 way cross-over study with a washout period of 1 week. A single dose of r-hirudin was administered intravenously in the morning of each study day. The subjects were randomized to receive 0.1, 0.2 or 0.4 mg/kg r-hirudin on each of the study days. The medication was administered intravenously over 2 minutes. The subjects remained in a recumbent position and a single lead ECG was recorded until 1 hour after each dosage. Breakfast and lunch were served 1 and 5 hours after medication. A light snack was served 7 hours after medication. 125 ml tap water was taken hourly up to 8 hours after medication; thereafter water was taken ad libitum by the subjects. Subjects remained in a supine position until 8 hours after medication. Subjects fulfilling all admission criteria were randomly allocated to one of six possible treatment sequences.

I	0.1 mg/kg, followed by 0.2 and 0.4 mg/kg body weight
II	0.1 mg/kg, followed by 0.4 and 0.2 mg/kg body weight
III	0.2 mg/kg, followed by 0.1 and 0.4 mg/kg body weight
IV	0.2 mg/kg, followed by 0.4 and 0.1 mg/kg body weight
V	0.4 mg/kg, followed by 0.1 and 0.2 mg/kg body weight
VI	0.4 mg/kg, followed by 0.2 and 0.1 mg/kg body weight

The study medication was labeled with the subject numbers. Each subject received only the sequence of the study medication carrying his/her number.

Formulation:
Substance: r-Hirudin
Dosage form: Vials containing 50 mg r-Hirudin as lyophilisate
0.1, 0.2 and 0.4 mg/kg administered over 2 minutes
Frequency: Once daily for each single dose
Manufacturer: Hoechst AG
Batch no.: 114011

Substance: Sterile water for injection
Dosage form: Ampules containing 20 ml sterile water for injection

Substance: Sodium Chloride 0.9%
Dosage form: Bags containing 200 ml sodium chloride 0.9%

50 mg r-hirudin was dissolved in 1 ml sterile water. The required dose, according to the weight of a particular volunteer was removed from this solution and diluted to 10 ml saline which was then injected intravenously over 2 minutes.

Subjects:

Eighteen healthy caucasian volunteers (9 male and 9 females) completed the study. Two subjects (#6 and 7, both females) dropped out of the study and were replaced (#19 and 20, both females). Subject 7 reported to the center during the second period with symptoms of bronchitis. She was receiving medication and was excluded from the study. Subject 6 had elevated eosinophils and was excluded from the study after completing the first phase.

Specimens:

r-Hirudin

2 ml blood samples were collected in citrate tubes for measurement of r-hirudin concentrations. (1.8 ml venous blood was added to 0.2 ml 3.8% sodium citrate). These specimens were taken before each medication and 10, 20, 30 minutes and 1, 1.5, 2, 3, 4, 5, 6 and 24 hours after each medication. The specimen taken before the first medication consisted of 8 ml citrate blood. (8 ml venous blood was added to 1 ml 3.8 % sodium citrate). Blood samples were immediately centrifuged and frozen.

TT, PTT:

Venous blood (4.5 ml) was collected in a citrate tube for measurement of TT and PTT. These specimens were taken before, 10 and 60 minutes as well as 2 and 5 hours after each medication. Analysis was done within 4 hours after collection.

Six ml blood was collected in a citrated tube to provide 3.0 ml plasma samples
was prepared by following the standard protocols
These samples were taken before each medication and at 10, 20, 30 minutes and 1, 1.5, 2, 3, 4, 5, 6, 24, 36, 48 and 72 hours after each medication. Aliquots of these samples were saved for future investigations of metabolism.

Antibody detection:

Blood (2 ml) was taken at screening and 3 weeks after the last medication for measurement of antibodies against r-hirudin. At least 0.5 ml serum was transferred into one labeled tube and stored frozen. Plasma preparation protocol

Urine collection and sampling schedule

The subjects emptied their bladders immediately before each dosing to provide a baseline sample. After medication fractionated urine specimens were collected as follows: 0-2, 2-4, 4-6, 6-8, 8-12, 12-24 and 24-48 hours after medication.

Fractionated urine volumes were recorded. Two 20 ml aliquots were taken from each specimen for r-hirudin determination All these aliquots were immediately frozen.

Results:

The pharmacokinetic evaluation of both plasma and urine data was performed on the concentrations determined in accordance with all previous studies (literature) of r-hirudin. These concentrations (free r-hirudin) were additionally

On each graph, a "line of perfect agreement" was also included to facilitate visual comparison of the two methods.

The following text summarizes the pharmacokinetic linearity of r-hirudin over a dose range of 0.1 mg/kg to 0.4 mg/kg.

Variable		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
C_{max} (ng/ml)	Mean	814	1467	2924
	SD	99.6	140	276
	CV%	12.2	9.56	9.45
	SEM	23.5	33.0	65.2
	Median	802	1476	2900
	n	18	18	18

Plasma r-hirudin pharmacokinetic variables (continued)

Variable		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
AUC_{last} (ng.h/ml)	Mean	650	1215	2419
	SD	118	147	259
	CV%	18.1	12.1	10.7
	SEM	27.7	34.7	61.0
	Median	625	1236	2446
	n	18	18	18
$AUC_{0-\infty}$ (ng.h/ml)	Mean	693	1269	2500
	SD	134	151	275
	CV%	19.3	11.9	11.0
	SEM	31.6	35.7	64.7
	Median	674	1297	2515
	n	18	18	18
$t_{1/2,a}$ (h)	Mean	0.20	0.20	0.24
	SD	0.09	0.08	0.09
	CV%	43.1	39.8	39.6
	SEM	0.02	0.02	0.02
	Median	0.19	0.17	0.22
	n	17	18	18

Parameter	1	2	3	4	5	6	7	8	9
CL (L/hr)	8.31 (22.5%)	10.89 (16.2%)	9.82 (19.3%)	3.67 (89.4%)	6.86 (46.8%)	5.56 (36.0%)	8.97 (33.7%)	8.14 (35.7%)	8.42 (28.3%)
V1 (L)	7.33 (27.0%)	6.51 (24.1%)	5.80 (20.3%)	6.36 (41.0%)	6.95 (35.7%)	12.61 (83.4%)	16.63 (38.3%)	15.15 (30.6%)	13.65 (45.1%)
Vss (L)	18.72 (20.6%)	17.27 (20.3%)	12.22 (16.4%)	18.03 (41.1%)	32.13 (98.9%)	29.86 (41.3%)	31.98 (28.1%)	22.41 (23.3%)	27.82 (24.5%)

1: elderly male and female subjects (70-80 yr)
2: healthy males
3: healthy males and females
4: male and female renally impaired
5: HAT patients
6: DVT patients
7: AMI patients/r-tPA lysis
8: AMI patients/SK lysis
9: unstable angina patients

Thus, age, gender, creatinine clearance, total dose and PK drug-drug interactions were observed to influence the pharmacokinetics of r-hirudin. Specifically, following effects were observed:

Gender: CL in females is 25.5% lower than males. V1 values in female is 18.8% lower than male.

Age: CL is inversely related to age

Creatinine clearance: CL is proportionally related to creatinine clearance

Drug-Drug Interaction: ASA and heparin treated patients had V1 values of 116% and 277% higher than other patients, respectively. Streptokinase coadministration reduces V2 by 52.6%.

PK-PD Evaluation of r-hirudin:

The effect of various lepirudin plasma concentrations on aPTT prolongation was studied in different patient populations including healthy volunteers (395 samples), thrombolyzed patients (1710 samples), HAT type II patients (580 samples) and other (1106 samples, mainly from DVT patients). Samples resulting in aPTT-ratios above 8.0 or below 0.5 were not taken into consideration.

aPTT-ratios appeared to be non-saturable, up to the highest lepirudin plasma concentrations of about 4 μg/ml.

For patients with adjunctive thrombolysis and HAT type II patients, the variability of aPTT ratios was high, compared to healthy volunteers and other patients. Thrombolyzed patients in addition showed higher aPTT elevations at the lowest lepirudin concentrations than non-

Plasma r-hirudin pharmacokinetic variables (continued)

Variable		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
$t_{1/2;b}$ (h)	Mean	1.21	1.27	1.33
	SD	0.46	0.29	0.22
	CV%	38.2	23.1	16.4
	SEM	0.11	0.07	0.05
	Median	1.17	1.27	1.32
	n	18	18	18
CL_{tot} (ml/min)	Mean	170	181	182
	SD	46.4	38.0	33.2
	CV%	27.2	21.0	18.2
	SEM	10.9	8.97	7.83
	Median	169	172	183
	n	18	18	18
CL_{tot} (ml/min/kg)	Mean	2.50	2.67	2.70
	SD	0.55	0.39	0.30
	CV%	21.8	14.6	11.0
	SEM	0.13	0.09	0.07
	Median	2.47	2.57	2.65
	n	18	18	18

Plasma r-hirudin pharmacokinetic variables (continued)

Variable		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
MT_{vss} (h)	Mean	1.46	1.55	1.52
	SD	0.42	0.27	0.15
	CV%	29.0	17.6	10.0
	SEM	0.10	0.06	0.04
	Median	1.40	1.61	1.50
	n	18	18	18
V_{ss} (l)	Mean	14.1	16.5	16.5
	SD	2.36	2.91	2.83
	CV%	16.7	17.6	17.2
	SEM	0.56	0.69	0.67
	Median	13.1	16.0	16.1
	n	18	18	18
V_{ss} (l/kg)	Mean	0.21	0.24	0.24
	SD	0.03	0.03	0.02
	CV%	15.1	12.6	9.51
	SEM	0.01	0.01	0.00
	Median	0.21	0.24	0.25
	n	18	18	18

The following text summarizes the urinary data for r-hirudin over a dose range of 0.1 mg/kg to 0.4 mg/kg.

Variable		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Ae(0-48h) (mg)	Mean	2.64	5.39	12.2
	SD	0.76	1.59	2.58
	CV%	28.9	29.5	21.1
	SEM	0.19	0.38	0.63
	Median	2.51	5.09	12.2
	n	16	17	17
Ae(0-48h) (% of dose)	Mean	38.1	39.4	45.5
	SD	9.55	10.2	6.91
	CV%	25.1	26.0	15.2
	SEM	2.39	2.48	1.68
	Median	37.6	37.6	45.4
	n	16	17	17
CL_{ren} (ml/min)	Mean	68.8	73.1	82.4
	SD	25.6	26.7	21.0
	CV%	37.2	36.5	25.4
	SEM	6.39	6.47	5.08
	Median	68.3	71.3	84.7
	n	16	17	17

The plasma and urine pharmacokinetic variables of r-hirudin, separated according to sex, are summarized below:

Mean (CV%) and range.

		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Plasma data:				
C_{max} (ng/ml)	Males	793(14.2)	1500(11.0)	2927(8.58)
	Females	835(10.4)	1433(7.56)	2922(10.8) 2481-3540

		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Plasma data:				
AUC_{last} (ng.h/ml)	Males	591(19.0)	1165(15.1)	2328(9.25)
	Females	708(13.5)	1265(7.73)	2511(11.0)
$AUC_{0-\infty}$ (ng.h/ml)	Males	629(18.8)	1220(14.9)	2407(9.50)
	Females	757(16.2)	1318(7.77)	2594(11.5)
$t_{1/2;a}$ (h)	Males	0.16(35.8)	0.19(43.8)	0.26(32.2)
	Females	0.26(34.0)	0.21(38.0)	0.22(48.2)
$t_{1/2;b}$ (h)	Males	0.99(22.2)	1.22(28.9)	1.35(18.6)
	Females	1.43(38.1)	1.33(17.1)	1.32(14.9)
CL_{tot} (ml/min)	Males	203(18.2)	208(15.7)	207(11.8)
	Females	138(20.9)	154(13.0)	157(12.0)
MT_{vss} (h)	Males	1.28(20.6)	1.46(18.7)	1.48(11.7)
	Females	1.64(30.0)	1.64(15.6)	1.56(8.02)
V_{ss} (l)	Males	15.3(15.6)	17.9(16.5)	18.3(14.3)
	Females	12.9(13.6)	15.1(14.3)	14.7(11.4)

Urine data:		0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
Ae(0-48h) (mg)	Males	2.99(23.8)	6.07(27.8)	14.5(10.0)
	Females	2.20(27.8)	4.62(24.2)	10.2(13.7)
Ae(0-48h) (% of dose)	Males	40.5(24.6)	41.2(30.4)	49.2(15.0)
	Females	35.0(25.1)	37.3(19.1)	42.2(11.1)
CL_{ren} (ml/min)	Males	82.0(29.3)	85.8(34.6)	100(12.4)
	Females	51.9(31.7)	59.0(23.2)	66.5(17.7)

The total clearance and the renal clearance in females were on average two-third (2/3) that of males. This differences could be partly accounted for by the differences in the body weight between males and females. Figure 7 and 8 show a semi-log plot of median plasma concentration versus time normalized to 0.4 mg /kg dose and median cumulative urinary r-hirudin excretion (as % of dose), respectively. Figure 9 show median plasma concentration versus time plots for males and females.

Pharmacodynamics

Coagulation variables

Partial thromboplastin time (PTT)

The mean values (CV%) and ranges of PTT (seconds) were as follows:

Sampling time (h)	0.1 mg/kg	0.2 mg/kg	0.4 mg/kg
0*	30.6(7.11)	31.1(6.28)	29.8(6.88)
0.167	77.1(11.0)	93.8(15.9)	111(8.81)
1	51.6(12.4)	59.7(12.5)	72.8(9.62)
2	42.3(11.1)	49.1(11.9)	57.5(11.1)
5	35.0(12.1)	37.4(8.10)	40.9(10.5)

*n=17

Figure 10 illustrate median aPTT values at different doses. There were no gender differences in aPTT response to doses 0.1 to 0.4 mg/kg.

Thrombin time (TT):

Thrombin time was only measured up to a value of 180 seconds. The value 180 was reported for individual TT-values to indicate TT-values longer than 180 seconds. Between 0.167 and 2 h, most subjects in the 0.2 and 0.4 mg/kg dose groups and some in the 0.1 mg/kg dose group maintained TT-values above 180 seconds. The mean baseline-values were 23.7 seconds (0.1 mg/kg), 23.9 seconds (0.2 mg/kg) and 23.1 seconds (0.4 mg/kg). By 5 h, no values had returned to baseline.

From the mean TT-values at 5 h, namely 35.3 (0.1 mg/kg), 56.2 (0.2 mg/kg) and 108 seconds (0.4 mg/kg), it appeared that the TT-values remained elevated longer with increasing doses.

Thrombin-hirudin-complex (THC):

The following figure illustrates the difference between r-hirudin plasma levels and plasma levels at 0.4 mg/kg iv dose.

Antibody Formation: Three weeks after drug administration specific IgG antibodies against hirudin were present in 6 subjects, none of whom had received r-hirudin before. These were still present in slightly reduced concentrations 3 months after the study

Conclusion:

1. The study showed that r-hirudin exhibits linear pharmacokinetics over the tested IV dose

range of 0.1 mg/kg to 0.4 mg/kg. r-Hirudin showed a mean clearance of 2.6 ml/min/kg and a mean half-life of about 75 min.

2. The total clearance and the renal clearance in females were on average two-third (2/3) that of males.
3. The aPTT values peaked around the same time as Cmax. The mean (SD) peak aPTT were 77.1(11.0), 93.8(15.9), 111(8.81) for 0.1, 0.2 and 0.4 mg/kg dose respectively. The aPTT returned to baseline values around 5.0 hr post administration. Also, there is a good correlation between r-hirudin plasma concentration and aPTT.
4. Thrombin time was only measured up to a value of 180 seconds. Therefore, the peak response could not be identified. At the end of 5 hours, TT did not come back to the baseline. TT-values remained elevated longer with increasing doses.
5. represented only small fraction of r-hirudin in plasma as plasma levels were 1/10 th of the free r-hirudin plasma levels.
6. Study showed a very good correlation between for detection of free-hirudin in plasma. However, the study report did not include the performance of quality control samples, thus, making it impossible to find out how the assay performed when the study plasma samples were analyzed.

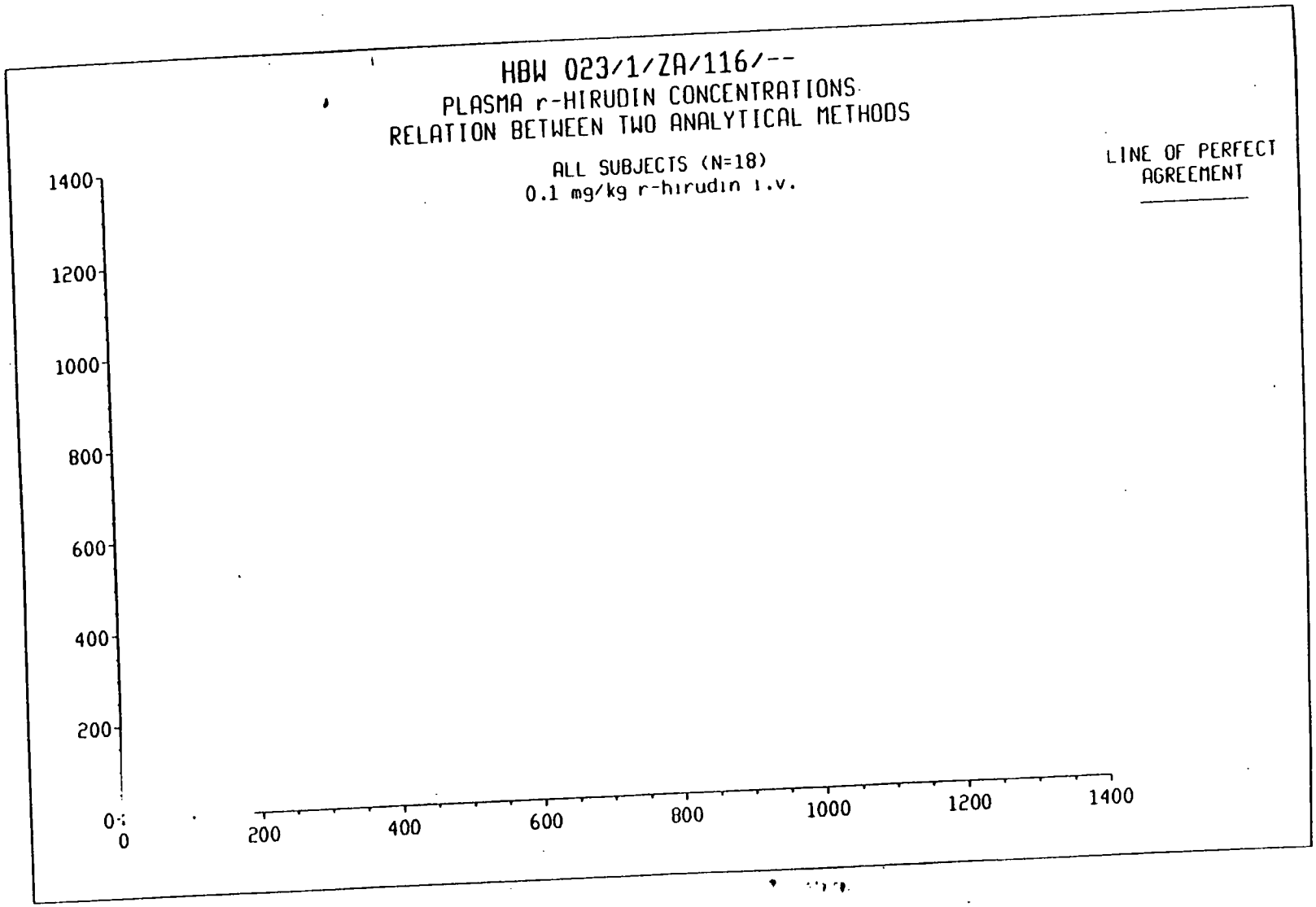

HBW 023/1/ZA/116/--
PLASMA r-HIRUDIN CONCENTRATIONS
RELATION BETWEEN TWO ANALYTICAL METHODS
ALL SUBJECTS (N=18)
0.1 mg/kg r-hirudin i.v.
LINE OF PERFECT AGREEMENT
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
1200
1400

Figure 1

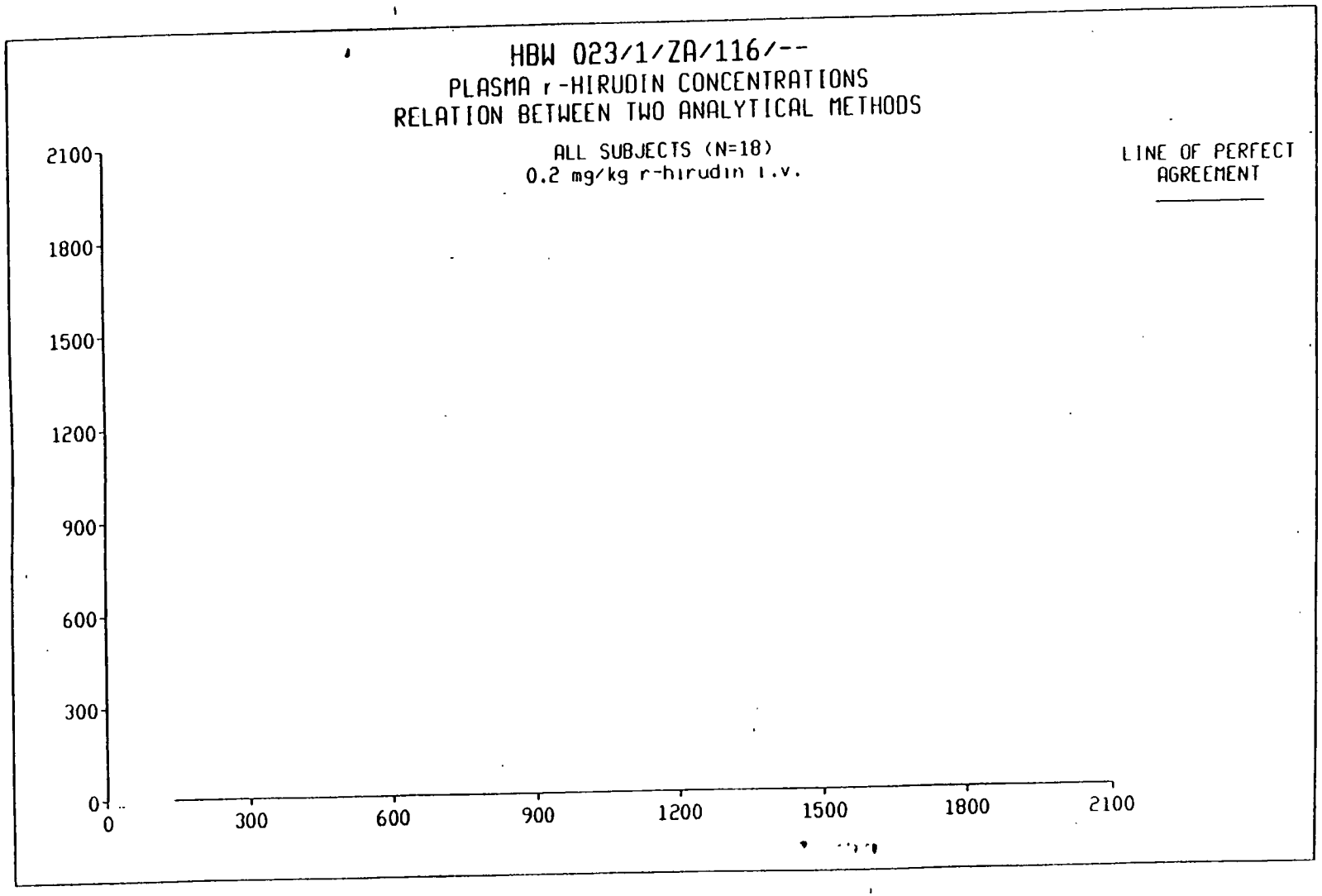

HBW 023/1/ZA/116/--
PLASMA r-HIRUDIN CONCENTRATIONS
RELATION BETWEEN TWO ANALYTICAL METHODS
ALL SUBJECTS (N=18)
0.2 mg/kg r-hirudin i.v.
LINE OF PERFECT AGREEMENT
2100
1800
1500
1200
900
600
300
0
0
300
600
900
1200
1500
1800
2100

Figure 2

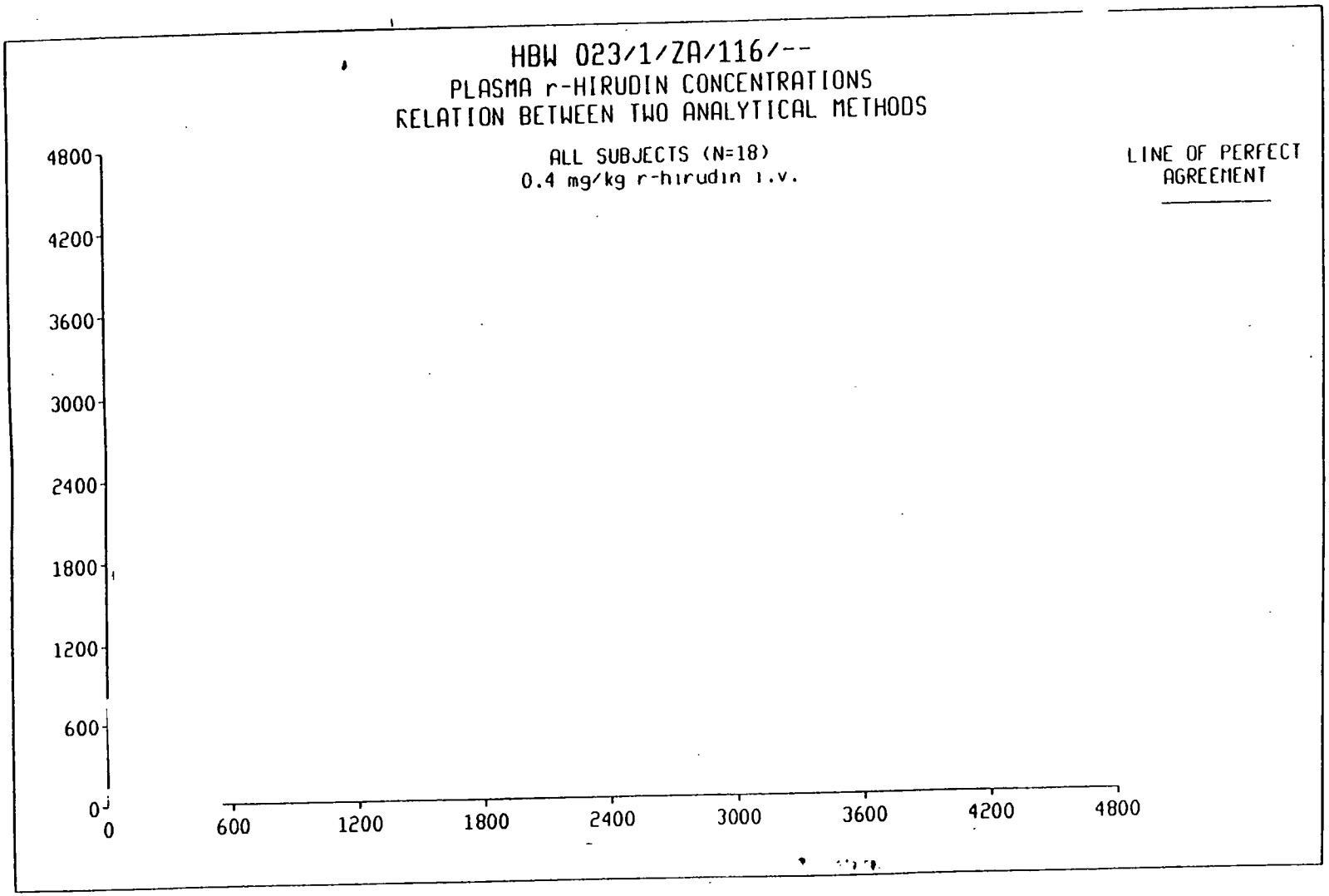

HBW 023/1/ZA/116/--
PLASMA r-HIRUDIN CONCENTRATIONS
RELATION BETWEEN TWO ANALYTICAL METHODS
ALL SUBJECTS (N=18)
0.4 mg/kg r-hirudin i.v.
LINE OF PERFECT AGREEMENT
4800
4200
3600
3000
2400
1800
1200
600
0
0
600
1200
1800
2400
3000
3600
4200
4800

Figure 3

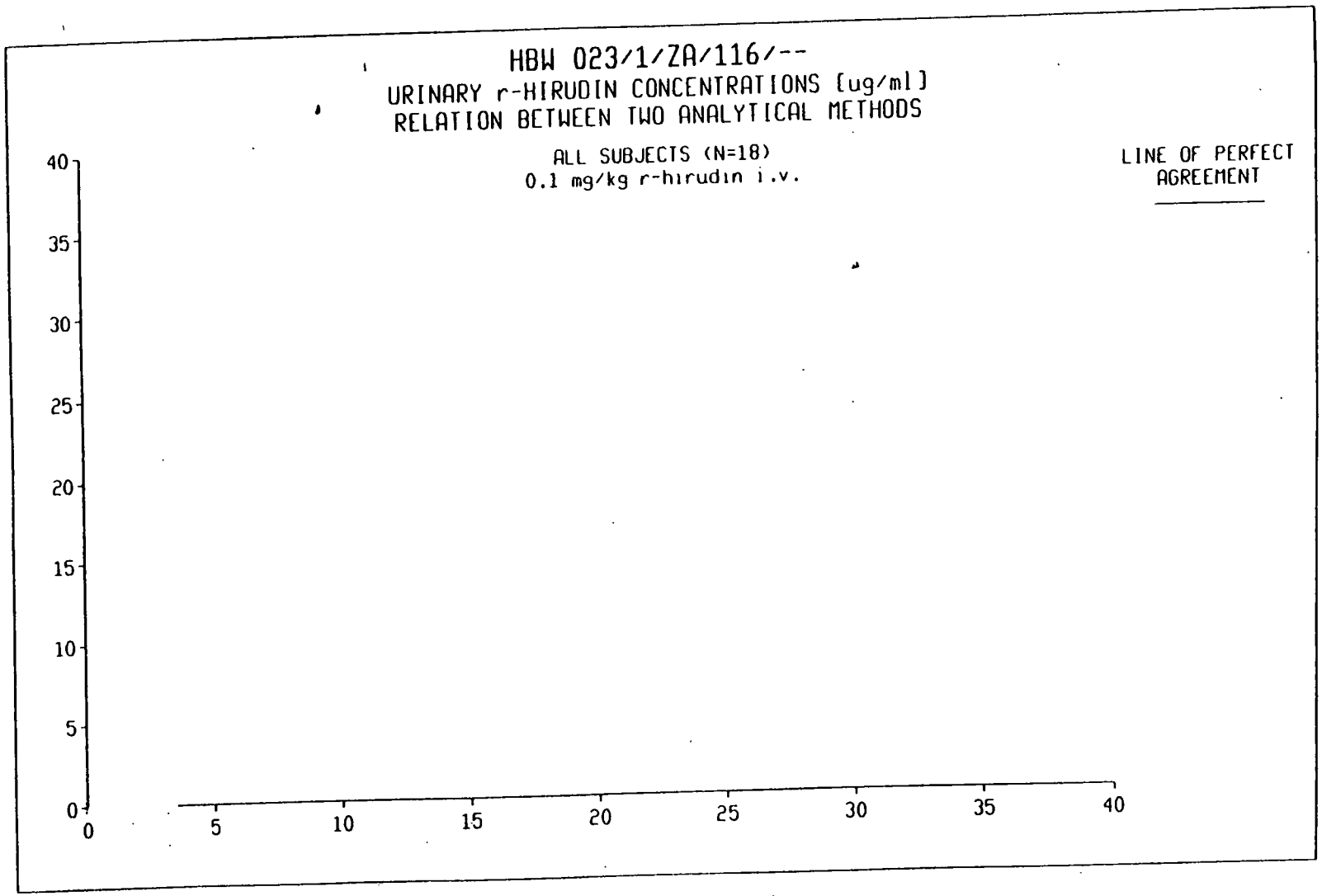

HBW 023/1/ZA/116/--
URINARY r-HIRUDIN CONCENTRATIONS [ug/ml]
RELATION BETWEEN TWO ANALYTICAL METHODS
ALL SUBJECTS (N=18)
0.1 mg/kg r-hirudin i.v.
LINE OF PERFECT AGREEMENT
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40

Figure 4

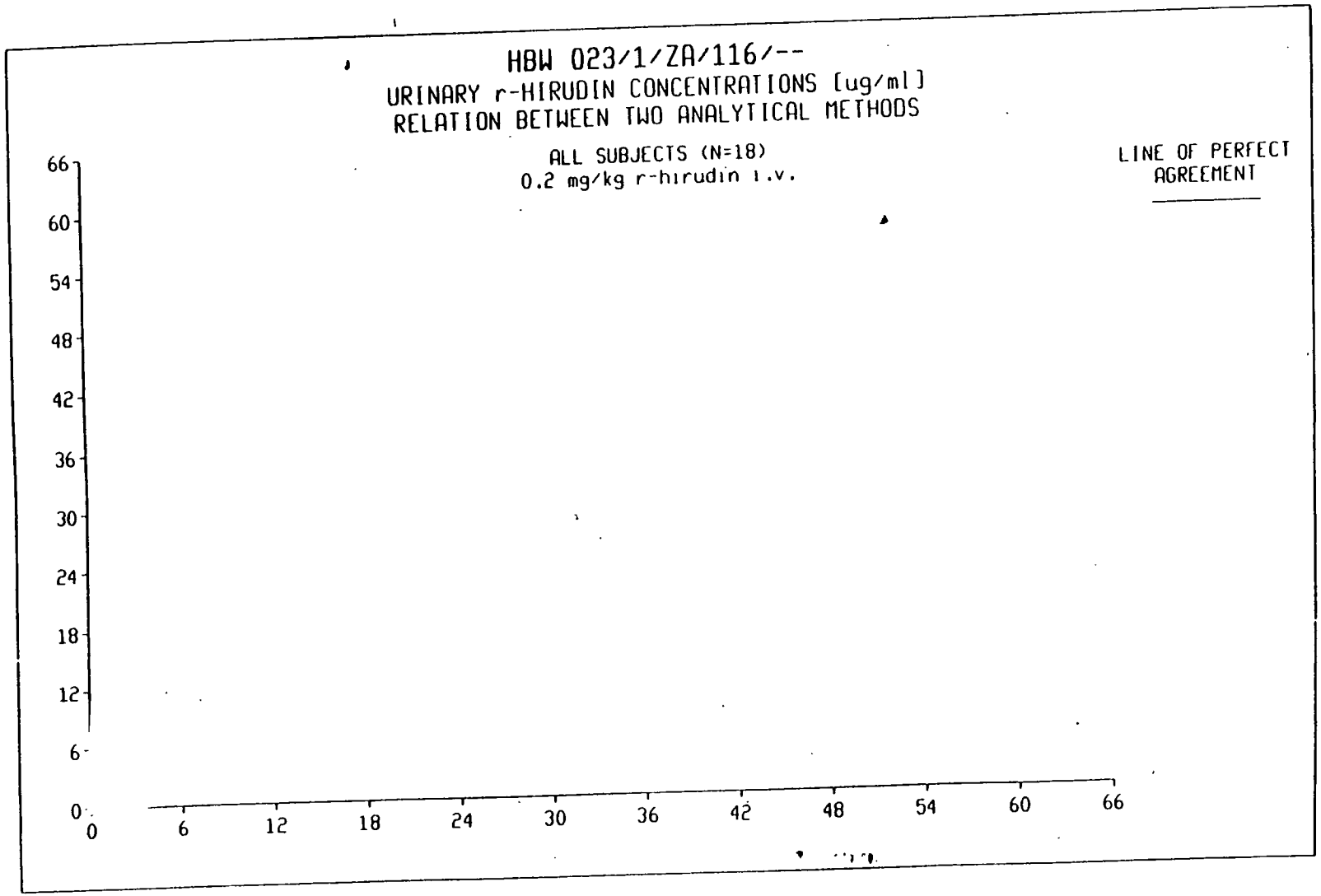
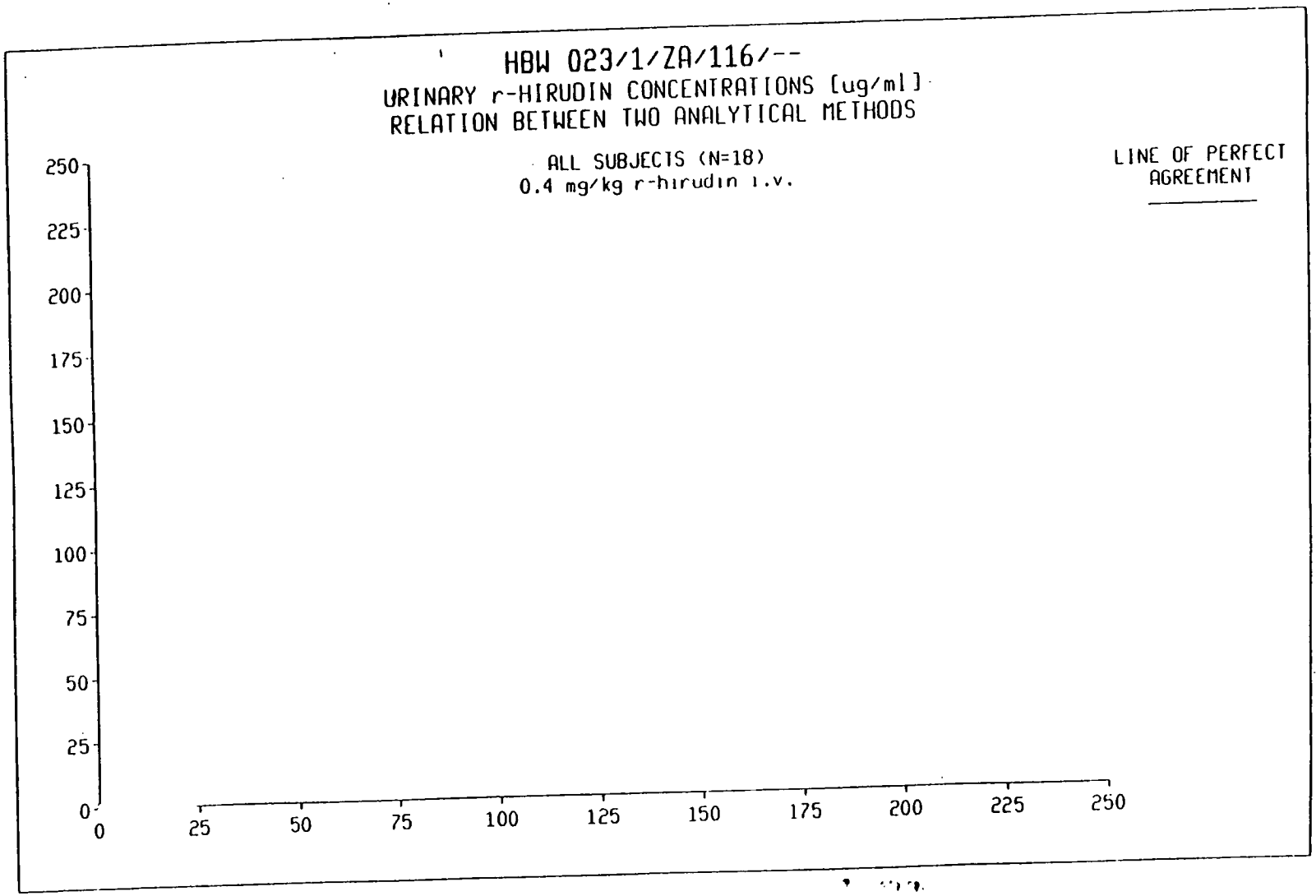

HBW 023/1/ZA/116/--
URINARY r-HIRUDIN CONCENTRATIONS [ug/ml]
RELATION BETWEEN TWO ANALYTICAL METHODS
ALL SUBJECTS (N=18)
0.2 mg/kg r-hirudin i.v.
LINE OF PERFECT AGREEMENT
66
60
54
48
42
36
30
24
18
12
6
0
0
6
12
18
24
30
36
42
48
54
60
66

Figure 5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URINARY r-HIRUDIN CONCENTRATIONS [ug/ml]
RELATION BETWEEN TWO ANALYTICAL METHODS
ALL SUBJECTS (N=18)
0.4 mg/kg r-hirudin i.v.
LINE OF PERFECT AGREEMENT
250
225
200
175
150
125
100
75
50
25
0
0
25
50
75
100
125
150
175
200
225
250

Figure 6

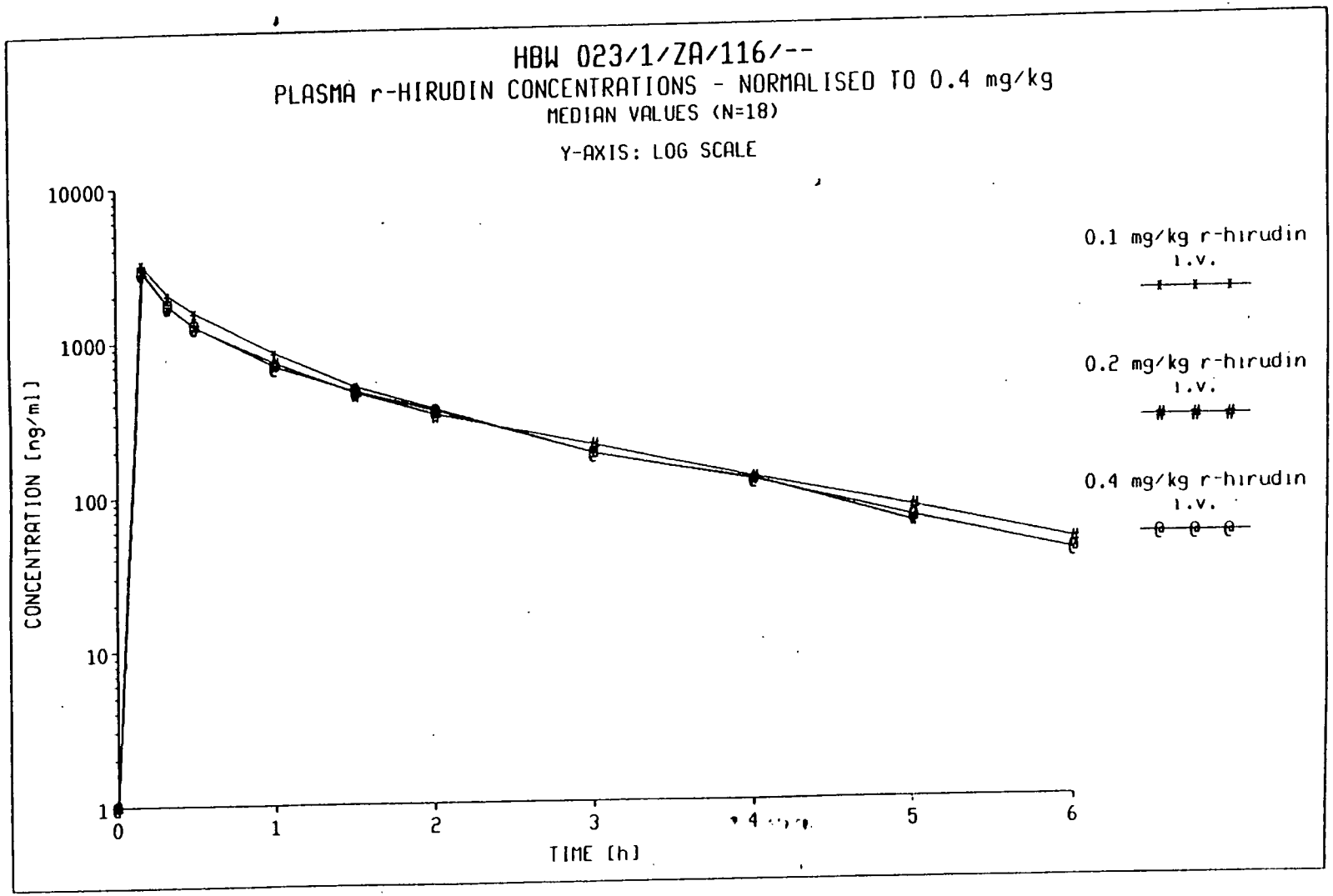
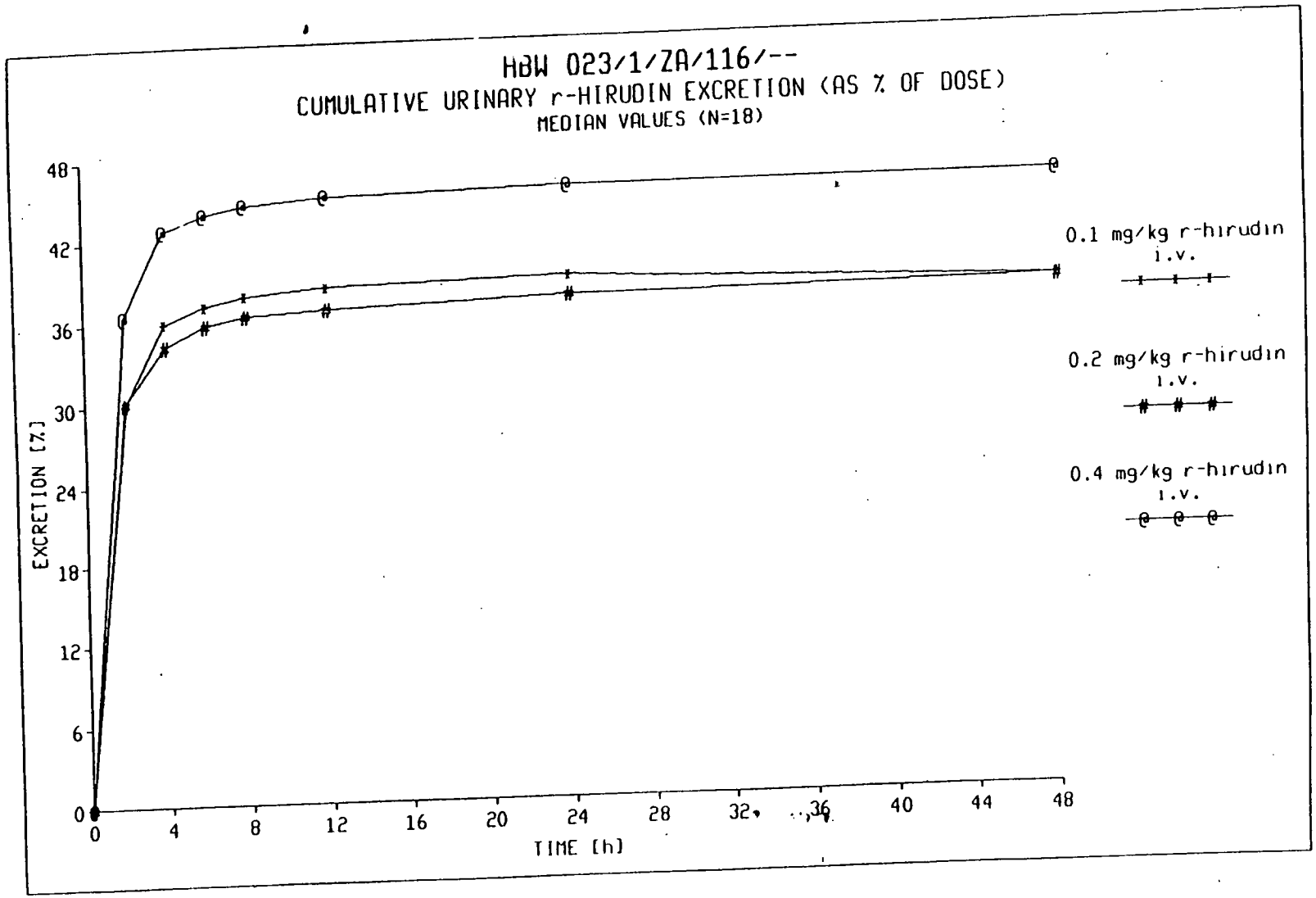
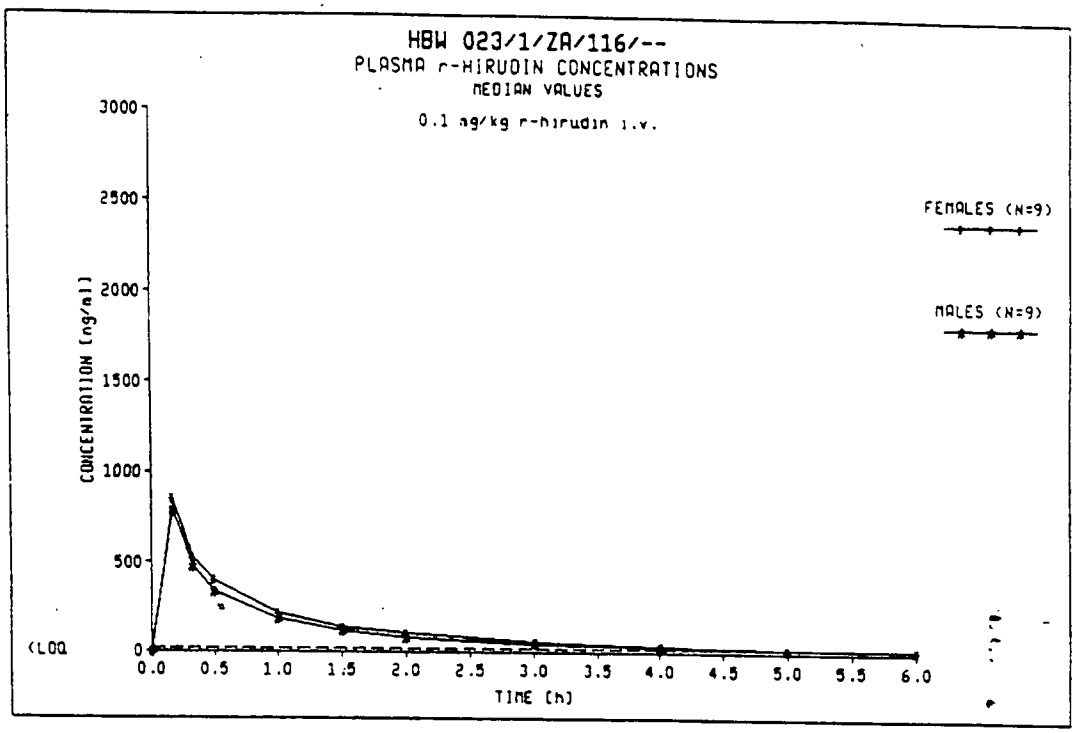


Figure 7



Figure

Figure 4a



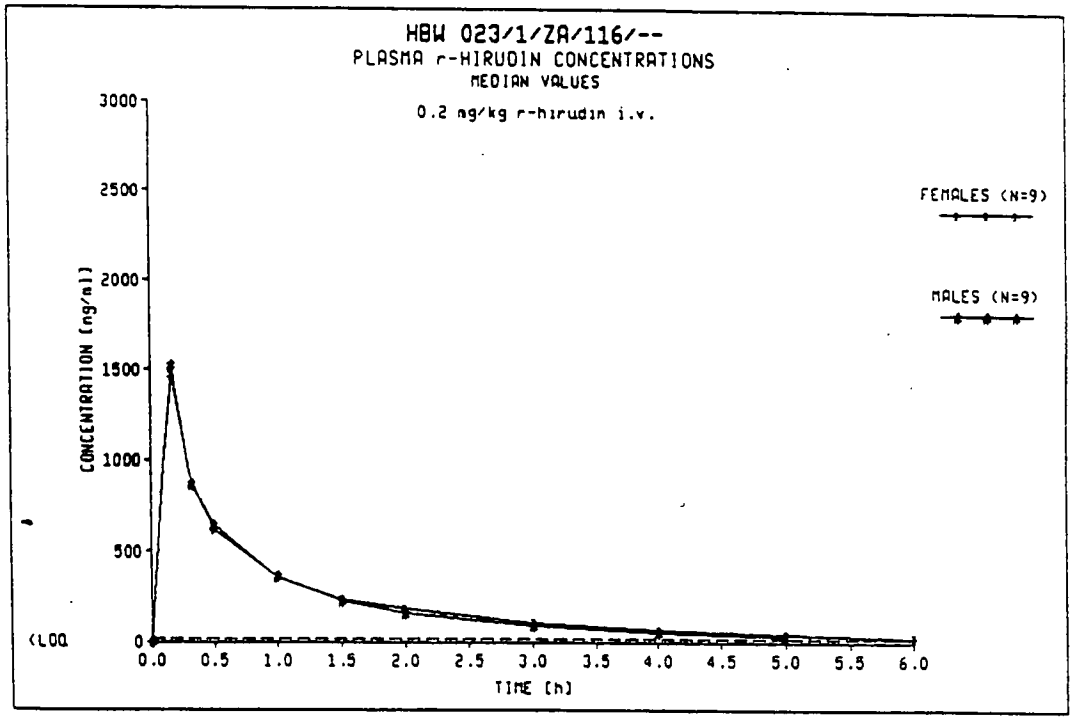
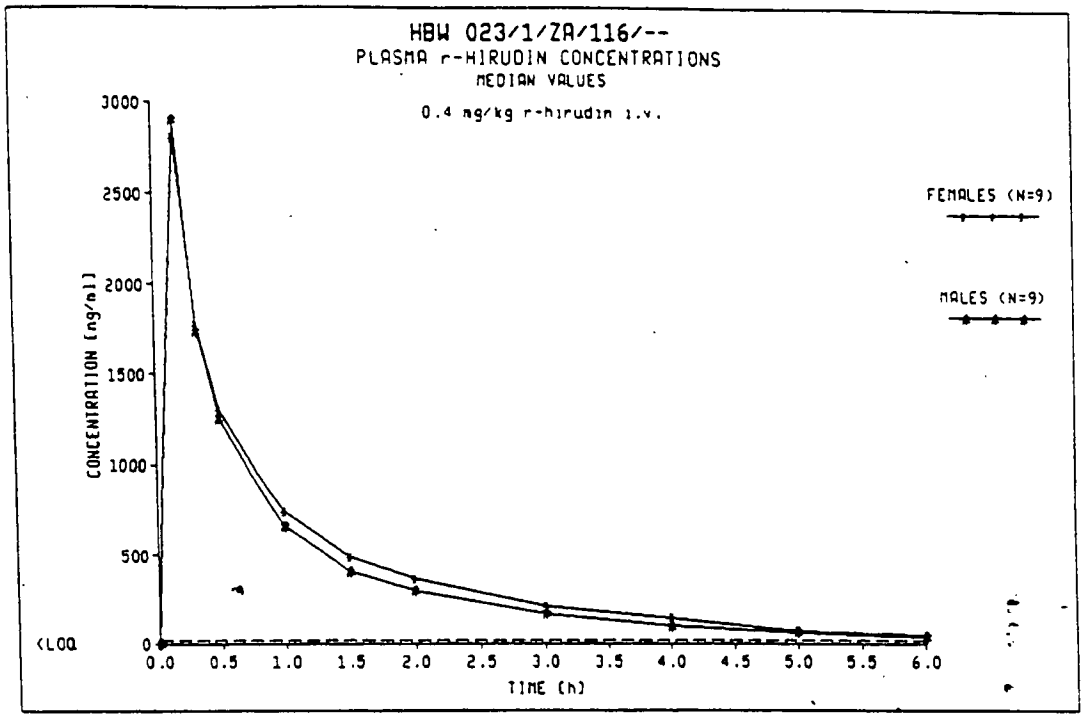


Figure 4b



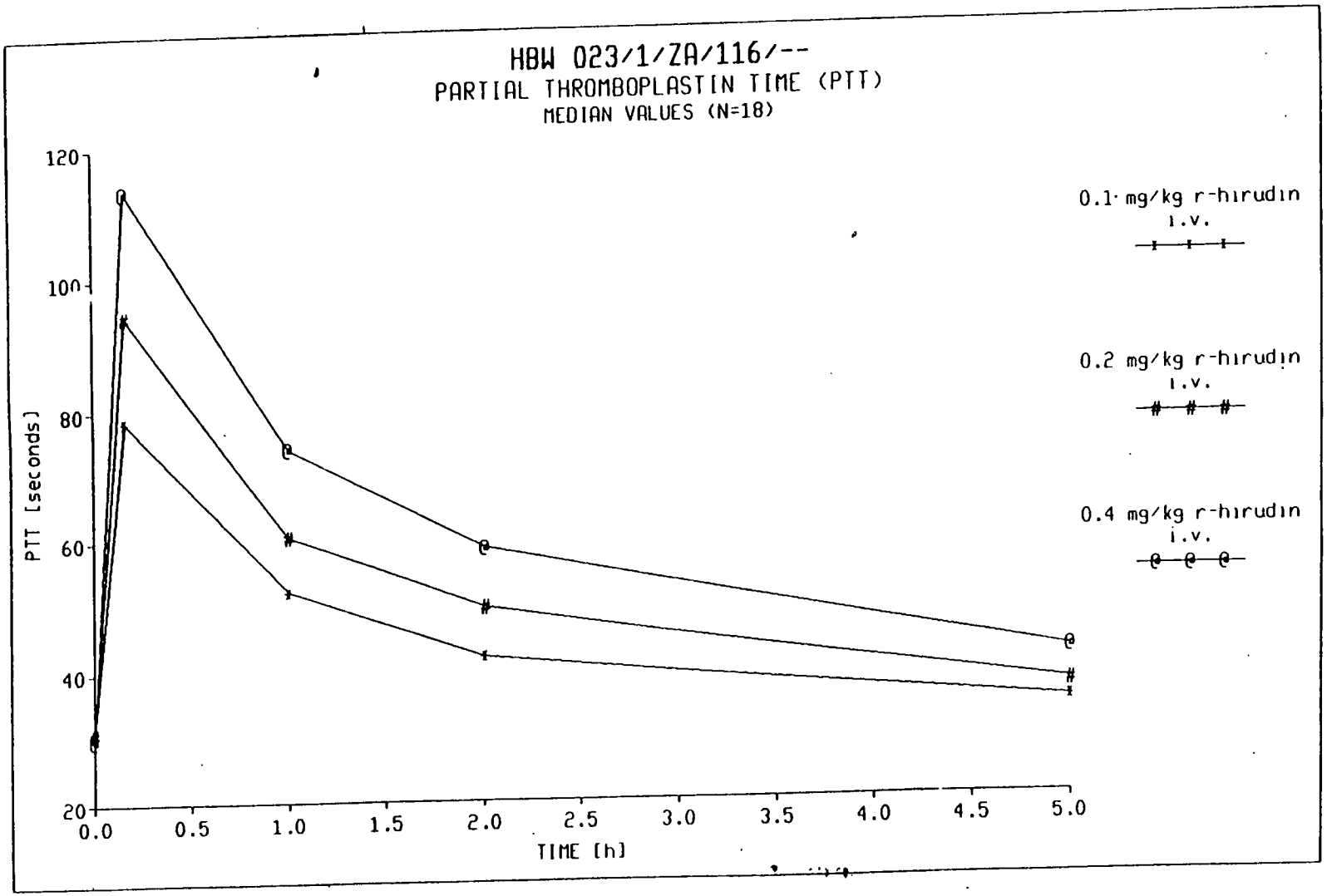


Figure 10

Tolerability, pharmacodynamics and pharmacokinetics of intravenous infusions (0.1, 0.15 and 0.2 mg/kg) of r-hirudin

Study: A4

Investigator and Site:

Hoechst Clinic, Dept. Of Pharmacology,
University of the Orange Free State, Bloemfontein, S. Africa
Study Dates: 01 to 02 -1990, Analytical Dates: 02 to 03 - 1990

Objectives:
To investigate the tolerance, pharmacodynamics and pharmacokinetics of single doses of 0.1, 0.15 and 0.2 mg/kg hirudin, given as IV infusion over six hours in healthy men.

Formulation:
r-hirudin lyophilized powder- 10 mg powder per vial

Study Design:
This was an open, parallel group study carried out in 15 healthy males (5 subjects per dose group). The following table shows the treatment assignment.

Dose (mg/kg)	Mean Age (yr)	Age range (yr)	Mean wt. (Kg)	Weight range (Kg)
0.1	20.4		73.8	
0.15	20	20	74.3	
0.2	20.8		81.8	

The demographic information did not include racial breakdown of the study population.

Specimens:
Blood for r-hirudin concentration: Two ml were collected at 0, 0.16, 0.5, 1, 2, 3, 4, 5, 6, 6.16, 6.33, 6.5, 7, 8, 9, 10, 11, 12 and 24 hours after commencement of infusion.

Blood for TT and aPTT profile: Coagulation profiles were measured before and at the following times after commencement of infusion: 0.16, 0.5, 1, 2, 3, 4, 5, 6, 8 and 24 hours.

Urine: Samples were collected and volumes were recorded at 0 and at 0-2, 2-4, 4-6 and 6-12 hours after commencement of infusion.

Assay:
Thrombin inhibition assay. Data on quality control samples were missing.

Results:

There were no drop-outs. The following table summarize the means, standard deviations and ranges of the pharmacokinetic parameters of hirudin

Parameters	0.1 mg/kg	0.15 mg/kg	0.2 mg/kg
Cmax (ng/ml)	111 (17.6)	203 (19.9)	246 (25.8)
Range			
$AUC_{0\text{-}6h}$ (ng.h/ml)	469 (68.4)	850 (76.1)	1109 (123)
Range			
$AUC_{6\text{-}11h}$ (ng.h/ml)	142 (32.0)	252 (53.8)	277 (49.0)
Range			
AUC (ng.h/ml)	612 (122)	1184 (152)	1446 (123)
Range			
t1/2 a (h)	0.07 (0.04)	0.16 (0.10)	0.12 (0.09)
Range			
t1/2 b (h)	1.10 (0.42)	1.98 (0.86)	1.36 (0.40)
Range			
Cltot (ml/min)	208 (43.7)	160 (32.6)	189 (14.7)
Range			
Vss (l)	17.1 (4.71)	17.0 (2.88)	16.0 (3.62)
Range			

Pharmacokinetic parameters in urine:

Parameters	0.1 mg/kg	0.15 mg/kg	0.2 mg/kg
Ae (0-12 h) mcg	2517 (608)	4380 (723)	5557 (2102)
Range			
Ae (0-12 h) (%dose)	34.1 (8.26)	39.4 (7.33)	33.4 (10.9)
Range			
Clren(0-12 h) ml/min	69.0 (18.6)	66.6 (14.8)	65.9 (22.6)
Range			

Figure 1 to 6 illustrate the pharmacokinetic profile and dose proportionality. Figure 7 to 11 illustrate the pharmacodynamic profile over the studied dose range.

Conclusion:

Pharmacokinetic parameters, viz. Cmax and AUC show a trend towards nonlinearity. The increase in mean Cmax and AUC was higher than This deviation from linearity however should be viewed in light of the parallel study design. It is difficult to make more conclusive statement on pharmacokinetic linearity for this study.

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ON ORIGINAL

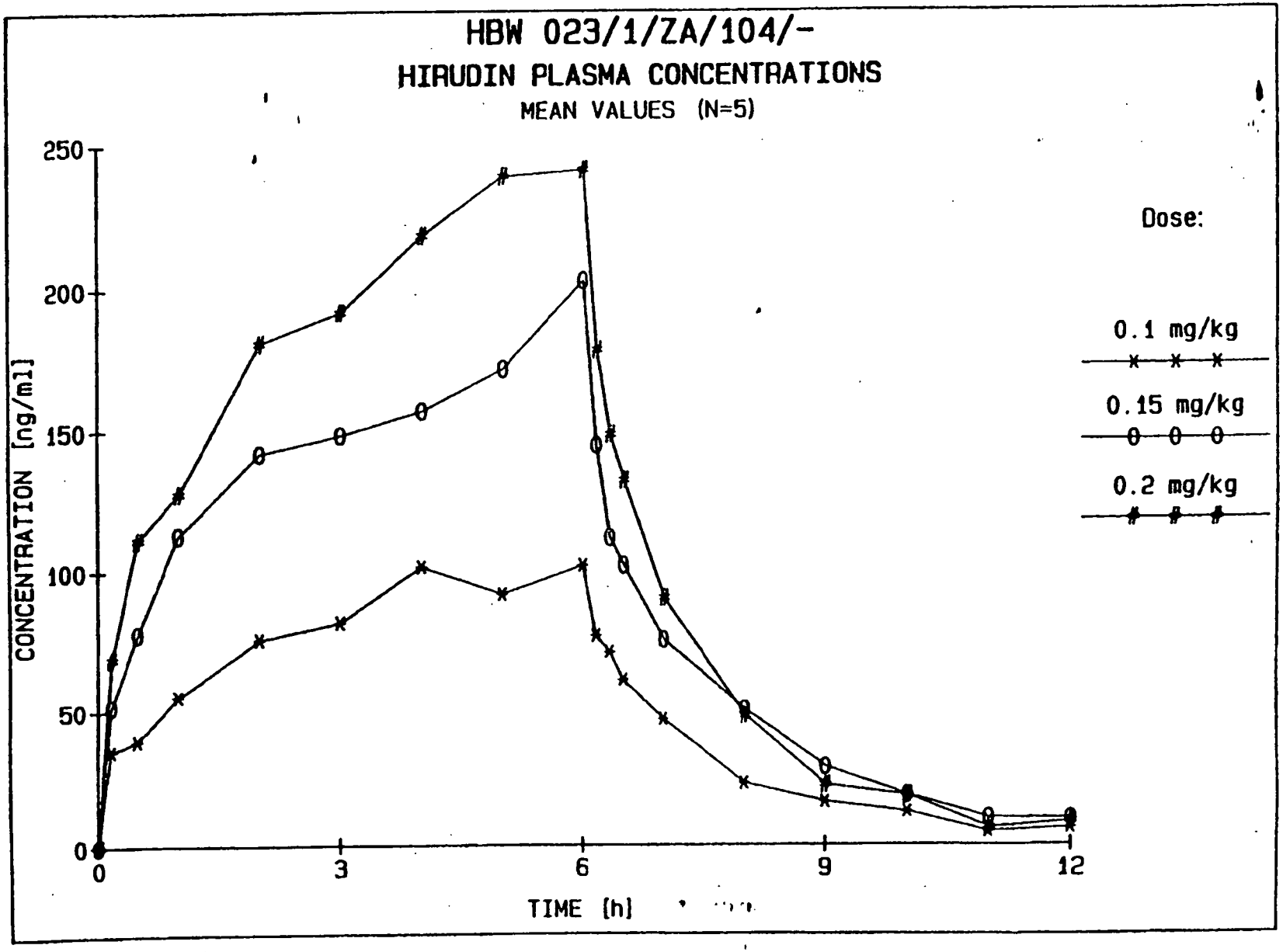

HBW 023/1/ZA/104/-
HIRUDIN PLASMA CONCENTRATIONS
MEAN VALUES (N=5)
CONCENTRATION [ng/ml]
250
200
150
100
50
0
0
3
6
9
12
TIME [h]
Dose:
0.1 mg/kg
0.15 mg/kg
0.2 mg/kg

Figure 1

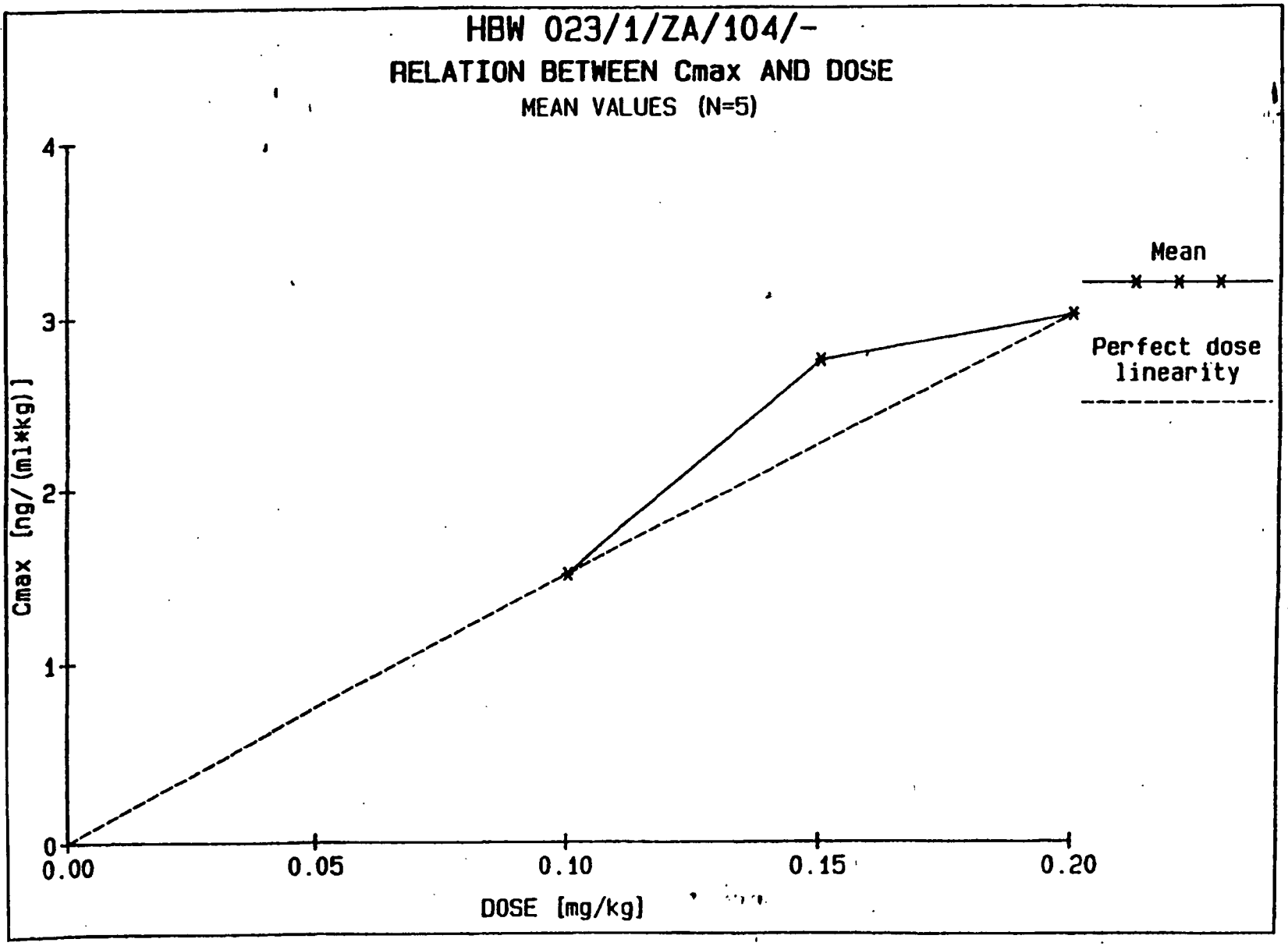

HBW 023/1/ZA/104/-
RELATION BETWEEN Cmax AND DOSE
MEAN VALUES (N=5)
Cmax [ng/(ml*kg)]
4
3
2
1
0
0.00
0.05
0.10
0.15
0.20
DOSE [mg/kg]
Mean
Perfect dose linearity

Figure 2

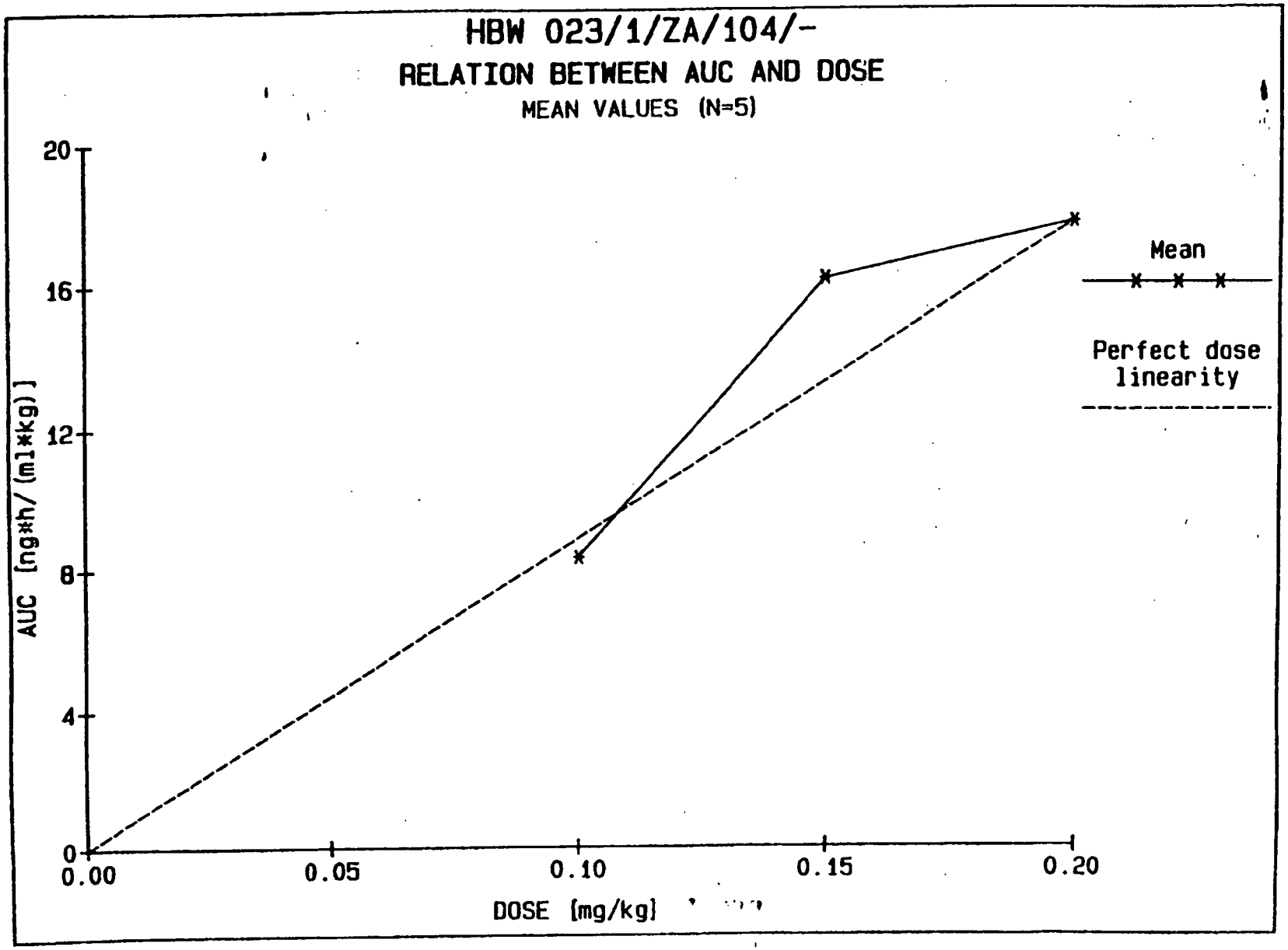
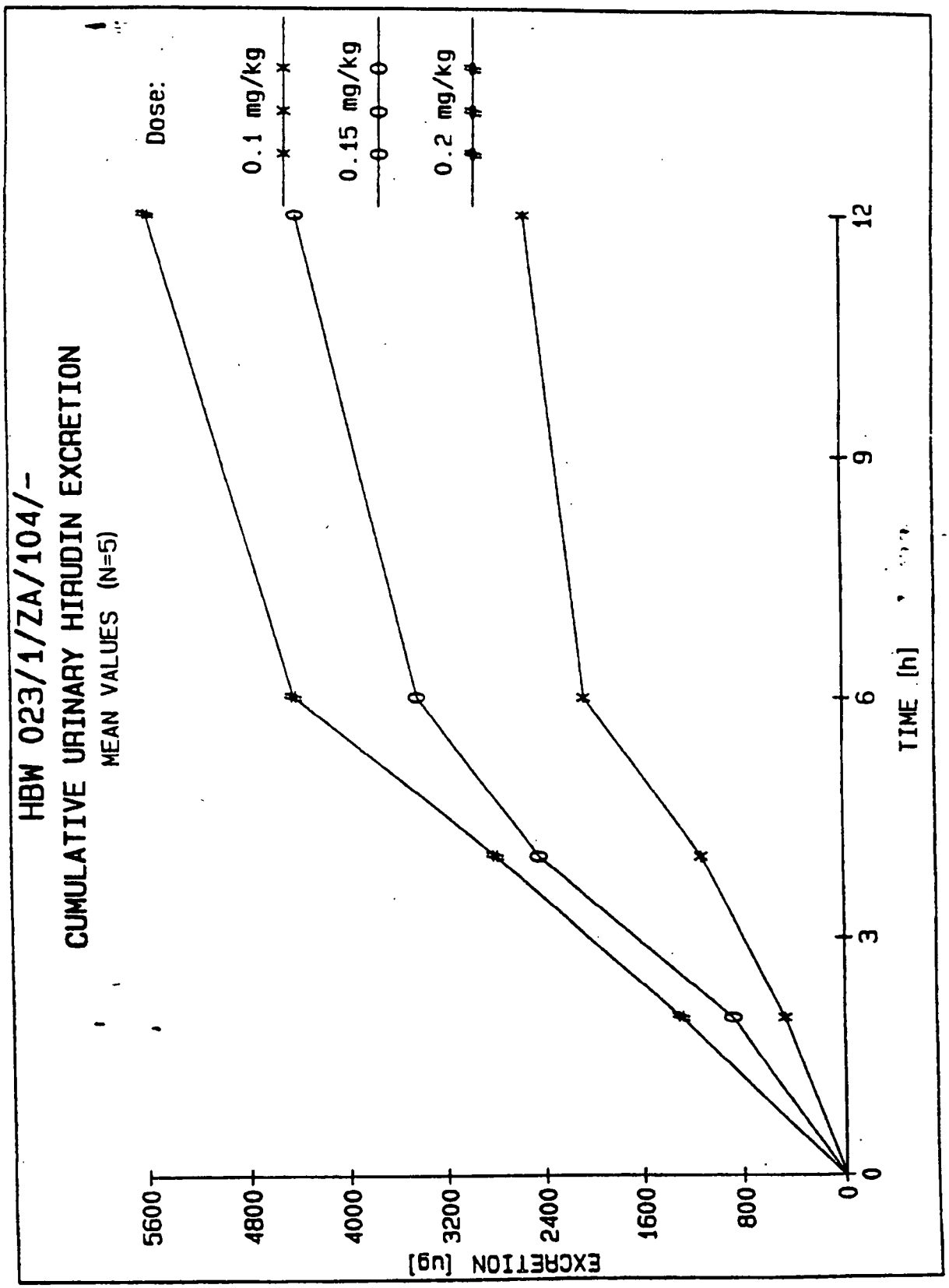

HBW 023/1/ZA/104/-
RELATION BETWEEN AUC AND DOSE
MEAN VALUES (N=5)
AUC [ng*h/(ml*kg)]
20
16
12
8
4
0
0.00
0.05
0.10
0.15
0.20
DOSE [mg/kg]
Mean
Perfect dose linearity

Figure 3

Figure 4


HBW 023/1/ZA/104/-
CUMULATIVE URINARY HIRUDIN EXCRETION
MEAN VALUES (N=5)
Dose:
0.1 mg/kg
0.15 mg/kg
0.2 mg/kg
EXCRETION [ug]
5600
4800
4000
3200
2400
1600
800
0
TIME [h]
3
6
9
12

064

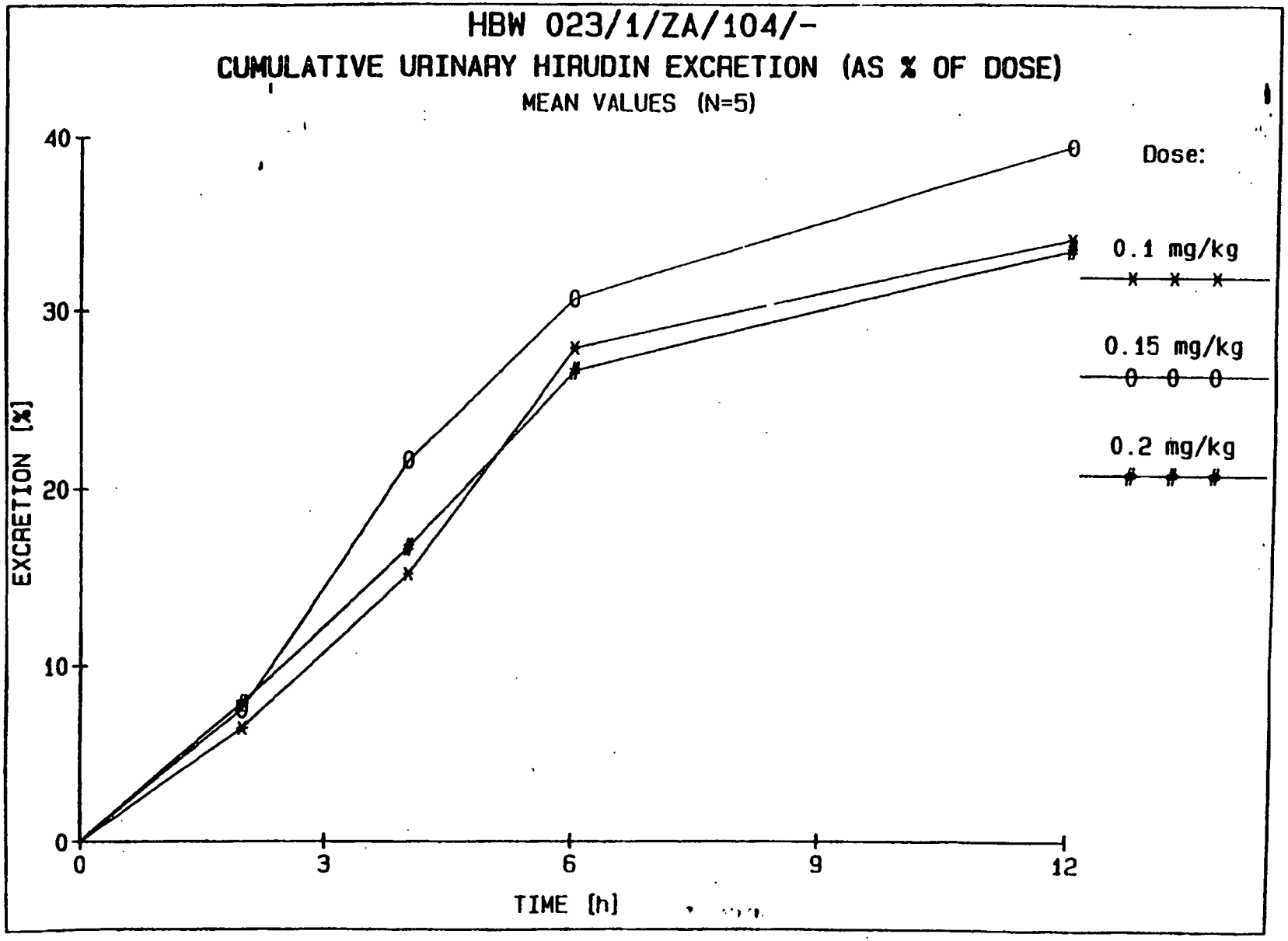

HBW 023/1/ZA/104/-
CUMULATIVE URINARY HIRUDIN EXCRETION (AS % OF DOSE)
MEAN VALUES (N=5)
EXCRETION [%]
TIME [h]
0
10
20
30
40
0
3
6
9
12
Dose:
0.1 mg/kg
0.15 mg/kg
0.2 mg/kg

Figure 5

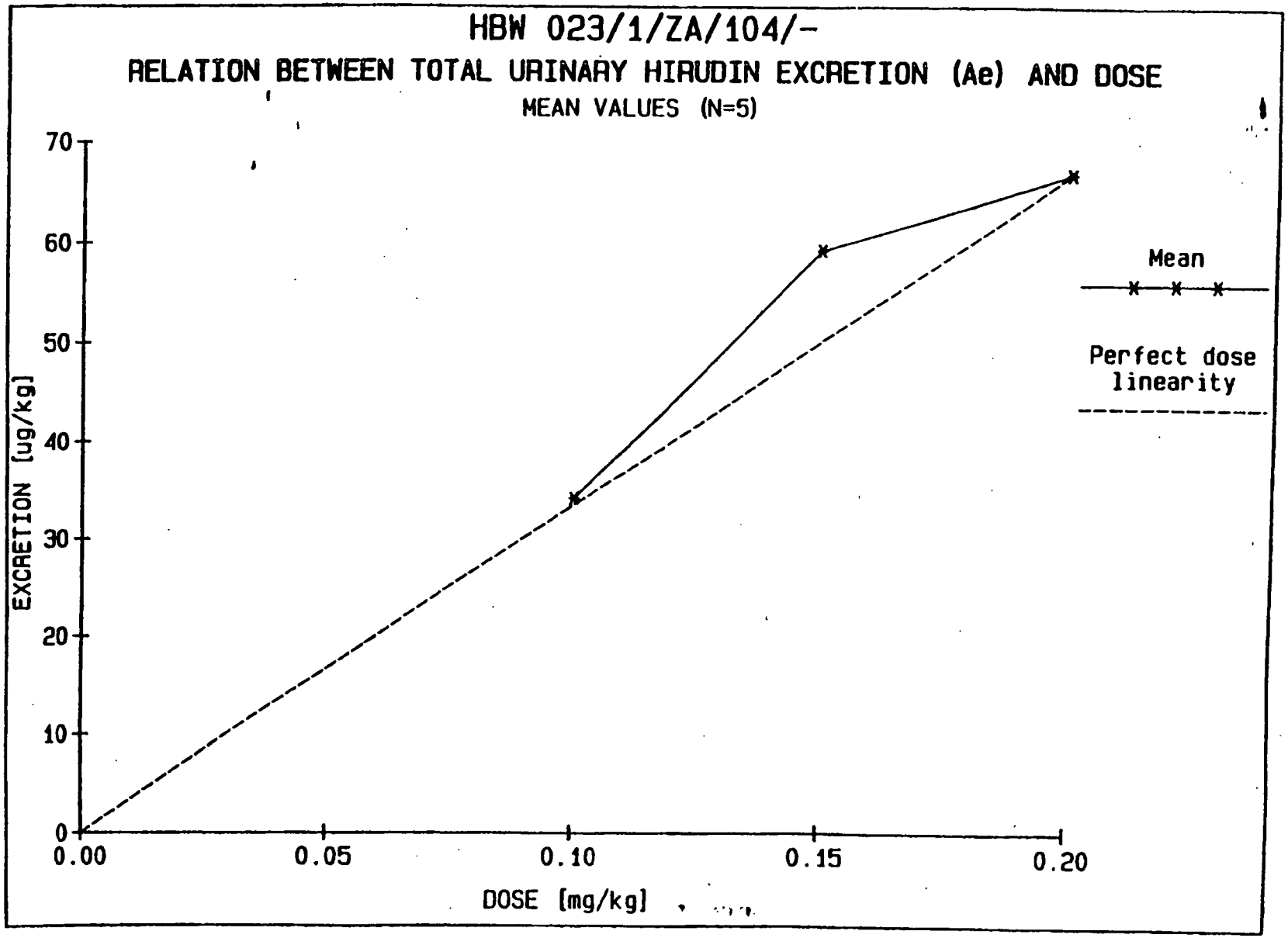

HBW 023/1/ZA/104/-
RELATION BETWEEN TOTAL URINARY HIRUDIN EXCRETION (Ae) AND DOSE
MEAN VALUES (N=5)
EXCRETION [ug/kg]
70
60
50
40
30
20
10
0
0.00
0.05
0.10
0.15
0.20
DOSE [mg/kg]
Mean
Perfect dose linearity

Figure 6

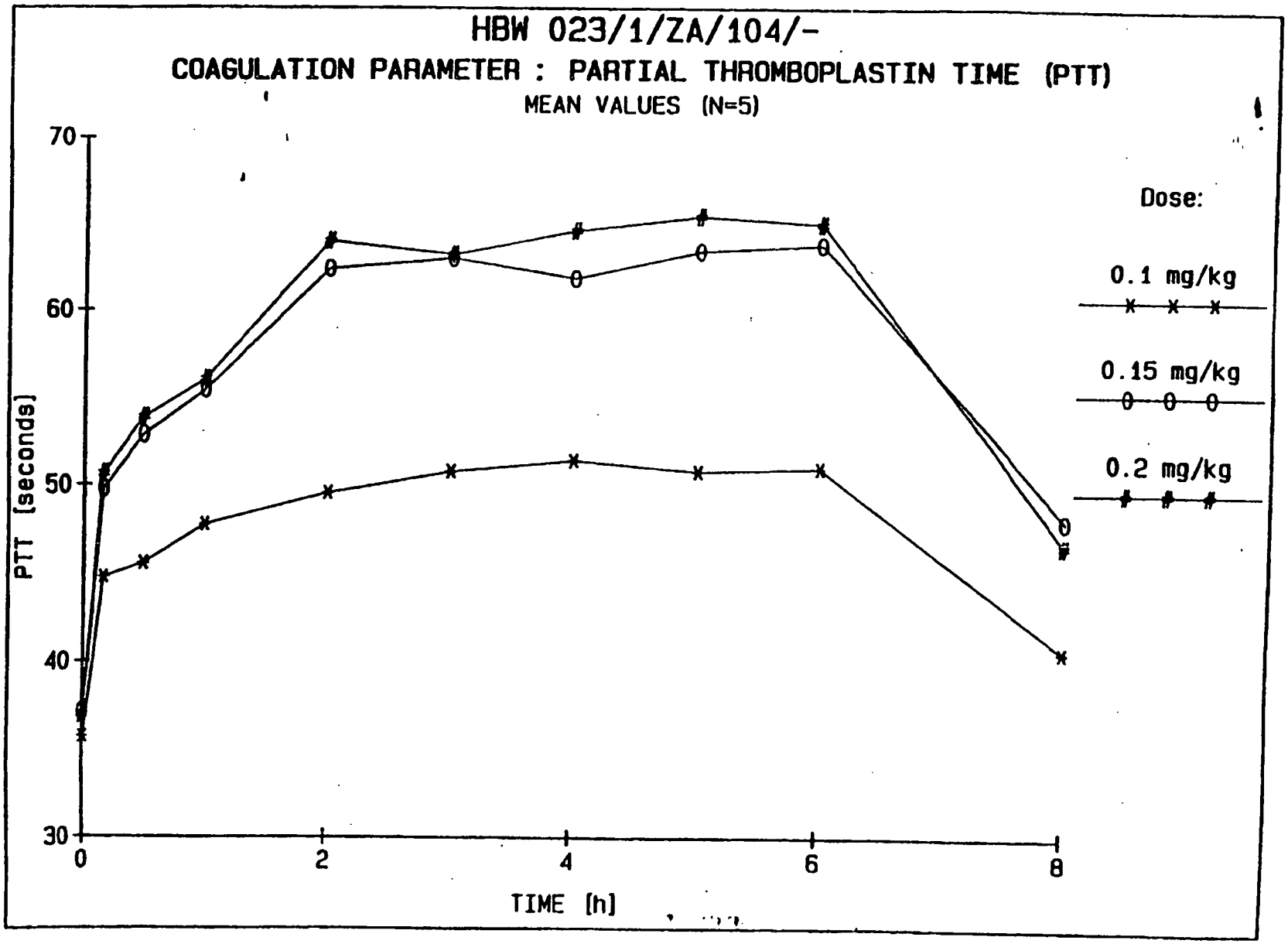

HBW 023/1/ZA/104/-
COAGULATION PARAMETER : PARTIAL THROMBOPLASTIN TIME (PTT)
MEAN VALUES (N=5)
PTT [seconds]
70
60
50
40
30
0
2
4
6
8
TIME [h]
Dose:
0.1 mg/kg
0.15 mg/kg
0.2 mg/kg

Figure 7

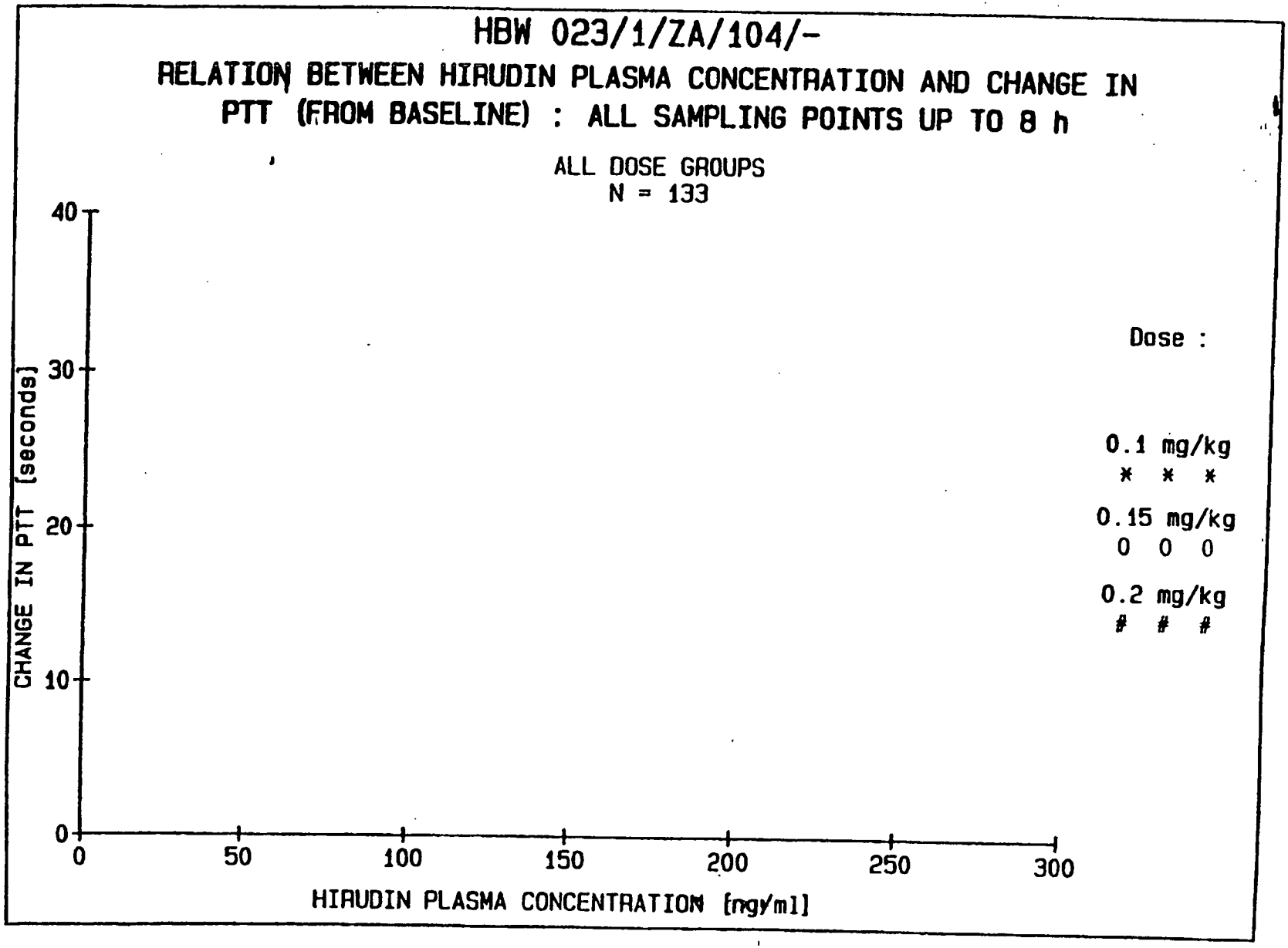

HBW 023/1/ZA/104/-
RELATION BETWEEN HIRUDIN PLASMA CONCENTRATION AND CHANGE IN PTT (FROM BASELINE) : ALL SAMPLING POINTS UP TO 8 h
ALL DOSE GROUPS
N = 133
CHANGE IN PTT [seconds]
40
30
20
10
0
0
50
100
150
200
250
300
HIRUDIN PLASMA CONCENTRATION [ng/ml]
Dose :
0.1 mg/kg
* * *
0.15 mg/kg
0 0 0
0.2 mg/kg
# # #

Figure 8

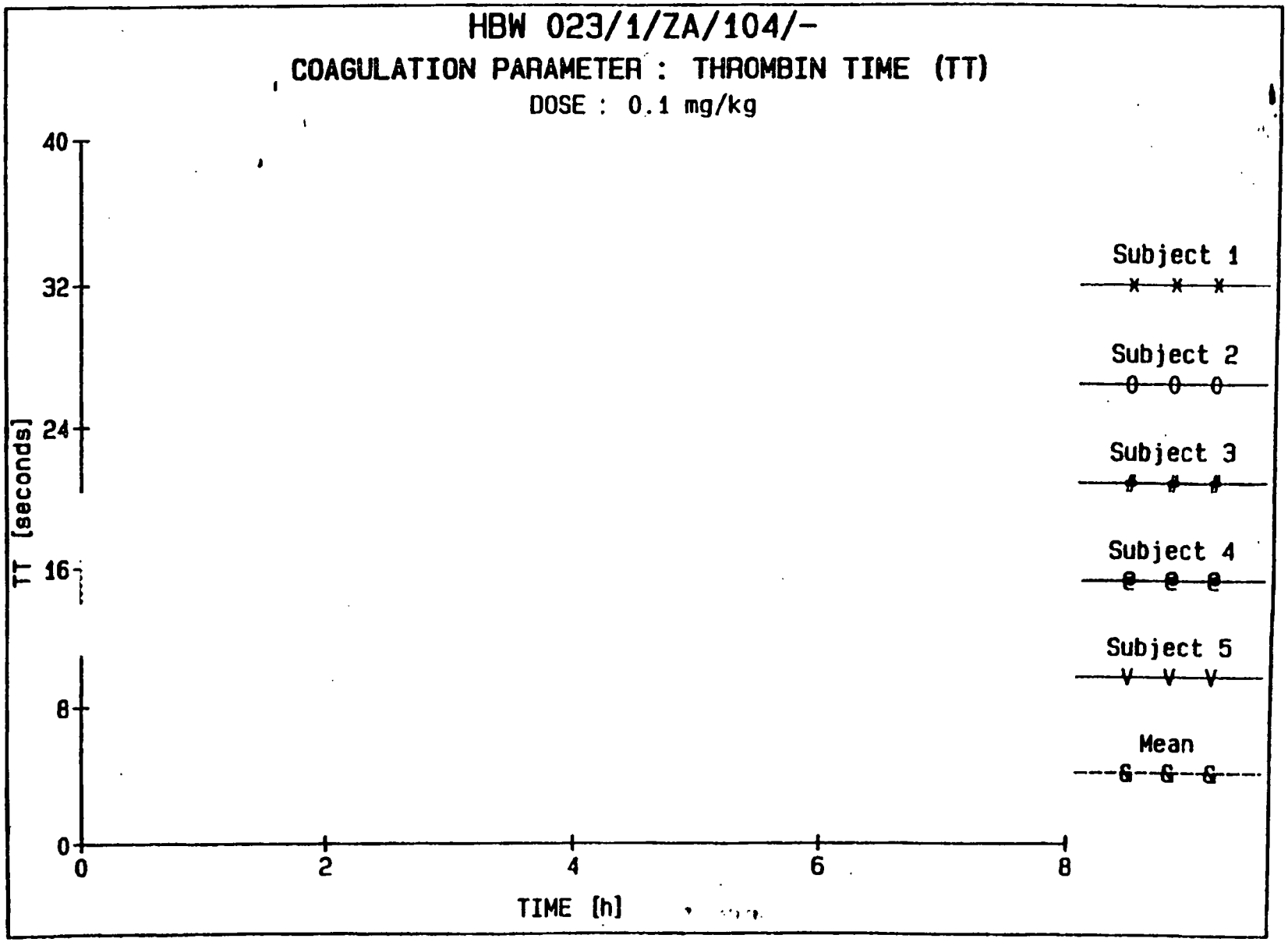

HBW 023/1/ZA/104/-
COAGULATION PARAMETER : THROMBIN TIME (TT)
DOSE : 0.1 mg/kg
TT [seconds]
40
32
24
16
8
0
0
2
4
6
8
TIME [h]
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Mean

Figure 12

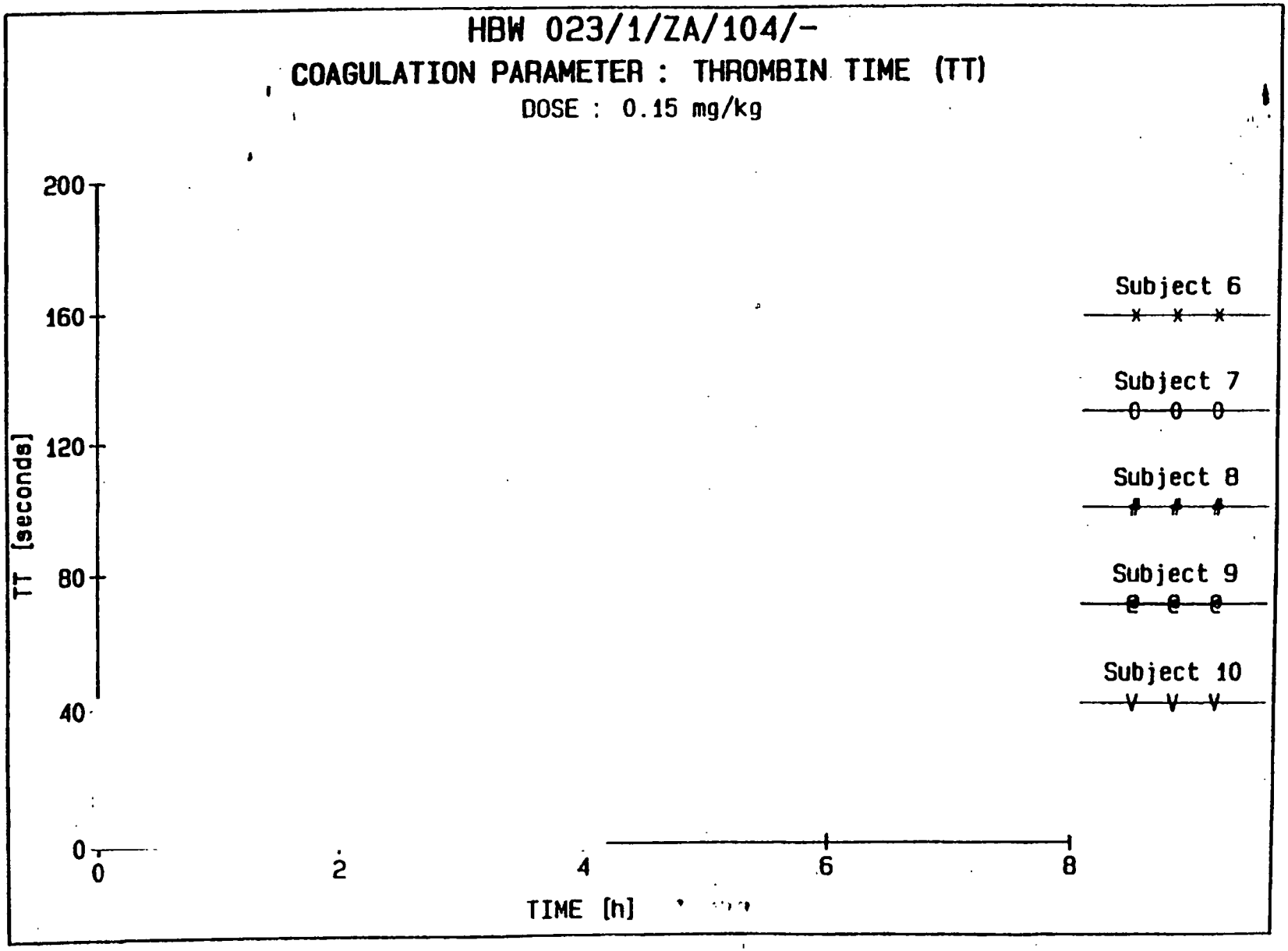

HBW 023/1/ZA/104/-
COAGULATION PARAMETER : THROMBIN TIME (TT)
DOSE : 0.15 mg/kg
TT [seconds]
200
160
120
80
40
0
0
2
4
6
8
TIME [h]
Subject 6
Subject 7
Subject 8
Subject 9
Subject 10

Figure 13

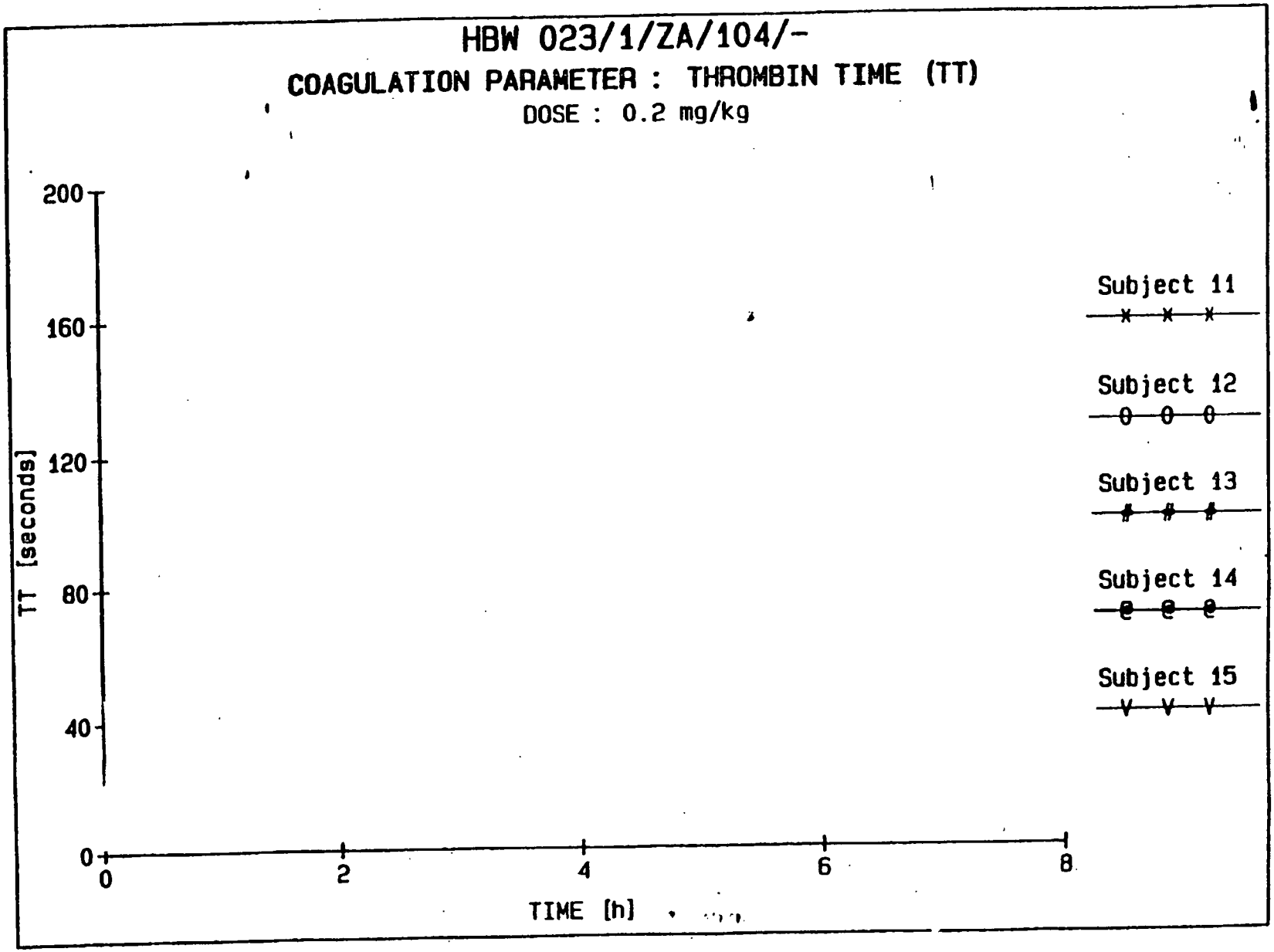

HBW 023/1/ZA/104/-
COAGULATION PARAMETER : THROMBIN TIME (TT)
DOSE : 0.2 mg/kg
TT [seconds]
200
160
120
80
40
0
0
2
4
6
8
TIME [h]
Subject 11
Subject 12
Subject 13
Subject 14
Subject 15

Figure 14

Pharmacokinetics and pharmacodynamics of subcutaneously administered hirudin (0.3 mg/kg) in healthy males and females

Study: A11

Investigator and Site:

Study Date: July-August, 1992
Analytical Date: October-December, 1992

Objective:
To investigate and compare the pharmacokinetics and pharmacodynamics of subcutaneously administered hirudin (0.3 mg/kg) in healthy males and females.

Study Design:
This was an open, controlled, single dose study with 14 healthy male and 14 healthy female volunteers All subjects received 0.3 mg/kg r-hirudin on the study day and completed all examinations.

The demographic information did not include racial breakdown of the study population.

Specimens:
Blood for r-hirudin concentration: Five ml were collected at 0, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hours after administration.

Blood for aPTT and TT: Coagulation profiles were measured before and at the following times after administration: 0.16, 0.33, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours.

Urine: Samples were collected and volumes were recorded at 0 and at 0-2, 2-4, 4-6, 6-8, 8-12, 12-18, 18-24 and 24-48 hours after administration.

Assay:
Data on quality control samples usually ran concomitantly with study samples were missing.

Results:

There were no drop-outs. Figure 1 to 5 show the pharmacokinetics and pharmacodynamics of r-hirudin administered subcutaneously.

The following table summarizes the mean, standard deviation and ranges of pharmacokinetic parameters of hirudin.

Plasma:

Parameters	Males	Female	Male* (mean)	Females* (mean)
Cmax (ng/ml) Range	329 (22.7)	346 (15.5)	2195	2191
tmax (h)# Range	2.5	2.00		
AUC (ng.h/ml) Range	1839 (17.1)	1691 (18.8)	1805	1945
t1/2a (h) Range	1.85 (17.5)	1.56 (14.7)		
t1/2z (h) Range	2.25 (24.9)	1.79 (14.1)	1.35	1.32
Cltot/F (ml/min) Range	225 (22.7)	191 (30.9)		

Urine:

Ae(0-48h) mg Range	8.92 (15.3)	5.06 (26.1)	10.9	7.65
$Ae_{(0\text{-}48h)}$ (%of dose) Range	37.4 (17.8)	27.6 (24.8)	49.2	42.2
CLren (ml/min) Range	82.7 (20.1)	51.2(28.4)	100	66.5

Median Value

* Means form study A3 (IV bolus) scaled to dose level 0.3 mg/kg

The following table summarizes the maximum aPTT and area under aPTT-time data pairs ($AUC_{0\text{-}24}$):

Parameter	Males	Females
Maximum aPTT (sec) Range	81.2(12.4)	78.1 (15.6)
$AUC_{0\text{-}24}$ (sec.h) Range	1293 (10.3)	1162 (10.8)

Comments:
Pharmacokinetic profile after SC administration of hirudin suggests a strong possibility of a flip-flop phenomenon, therefore, the terminal half-life is probably confounded by absorption process/rate.

Pharmacodynamically, the IV route shows peak aPTT at about 10 min post administration where as peak aPTT for SC route shows up around 2.5 hours.

Conclusion:
The rate of absorption of r-hirudin was higher for females than for males. The apparent total clearance for females was slightly lower than for males. This difference probably arose from the smaller renal clearance in females which in turn may be attributed to a slightly lower renal function (as judged by creatinine clearance) known to occur in female for a given weight and age.

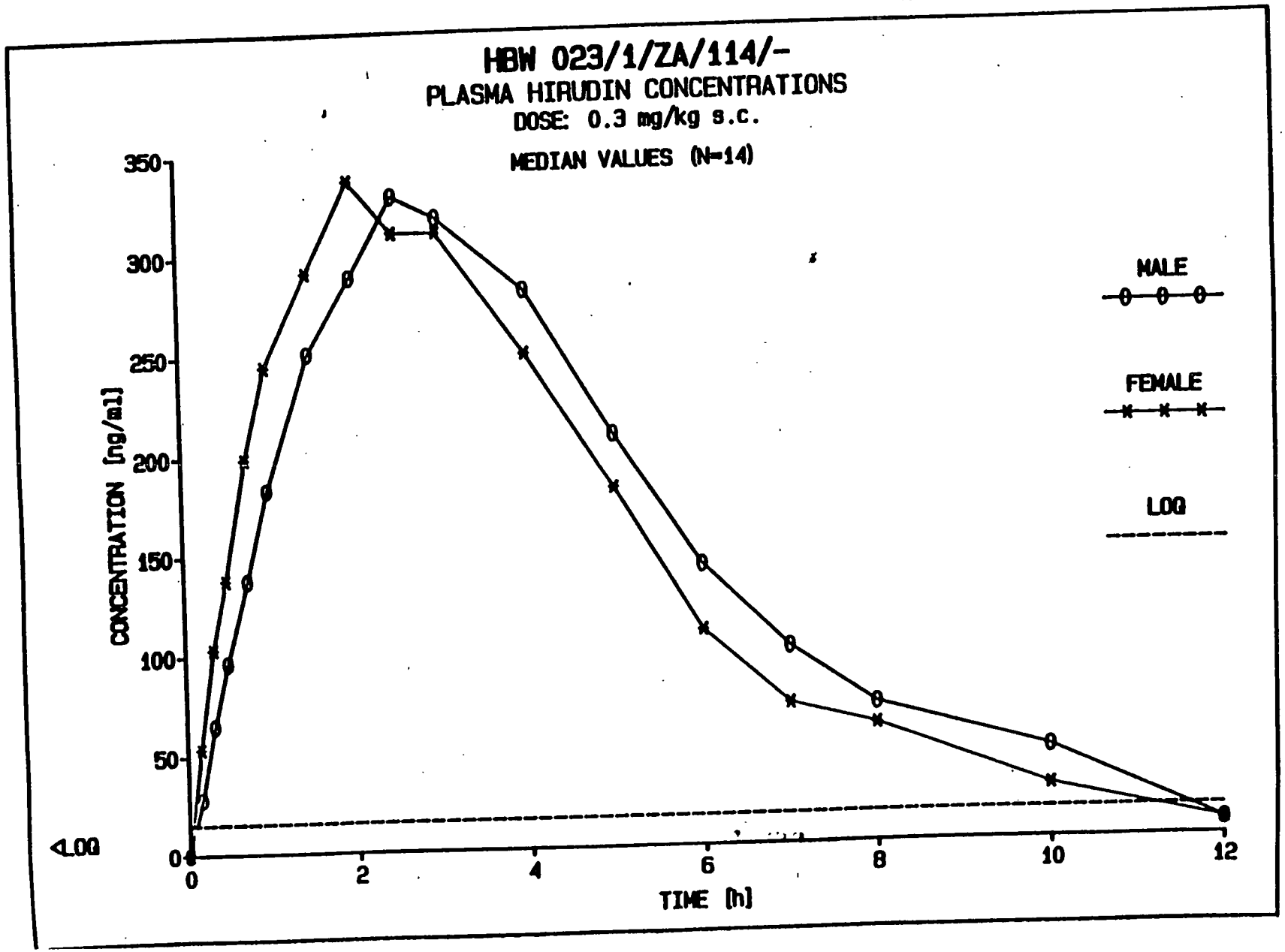

HBW 023/1/ZA/114/-
PLASMA HIRUDIN CONCENTRATIONS
DOSE: 0.3 mg/kg s.c.
MEDIAN VALUES (N=14)
CONCENTRATION [ng/ml]
350
300
250
200
150
100
50
0
<LOQ
0
2
4
6
8
10
12
TIME [h]
MALE
FEMALE
LOQ

Figure 1

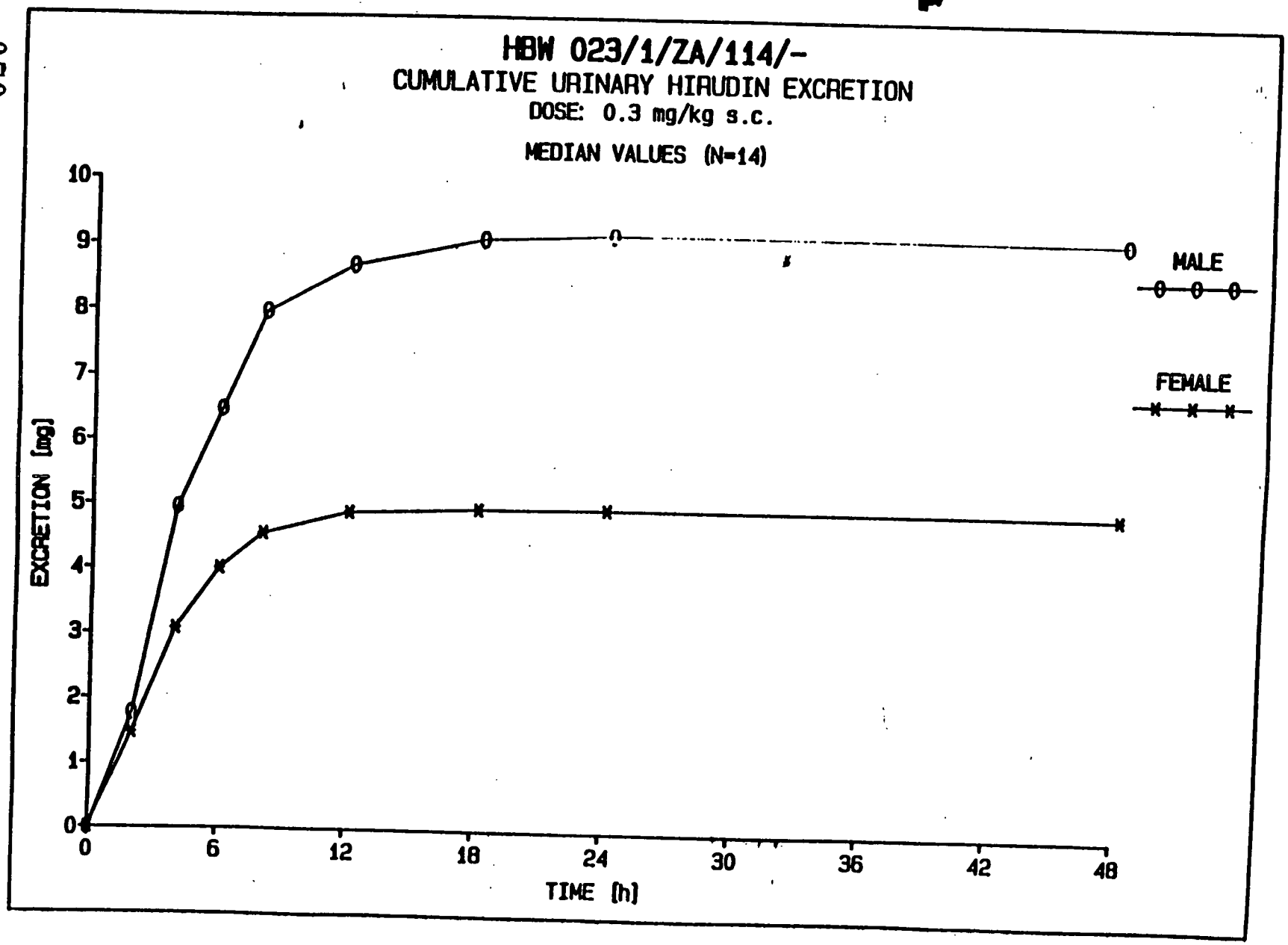

HBW 023/1/ZA/114/-
CUMULATIVE URINARY HIRUDIN EXCRETION
DOSE: 0.3 mg/kg s.c.
MEDIAN VALUES (N=14)
EXCRETION [mg]
TIME [h]
0
1
2
3
4
5
6
7
8
9
10
0
6
12
18
24
30
36
42
48
MALE
FEMALE

Figure 2

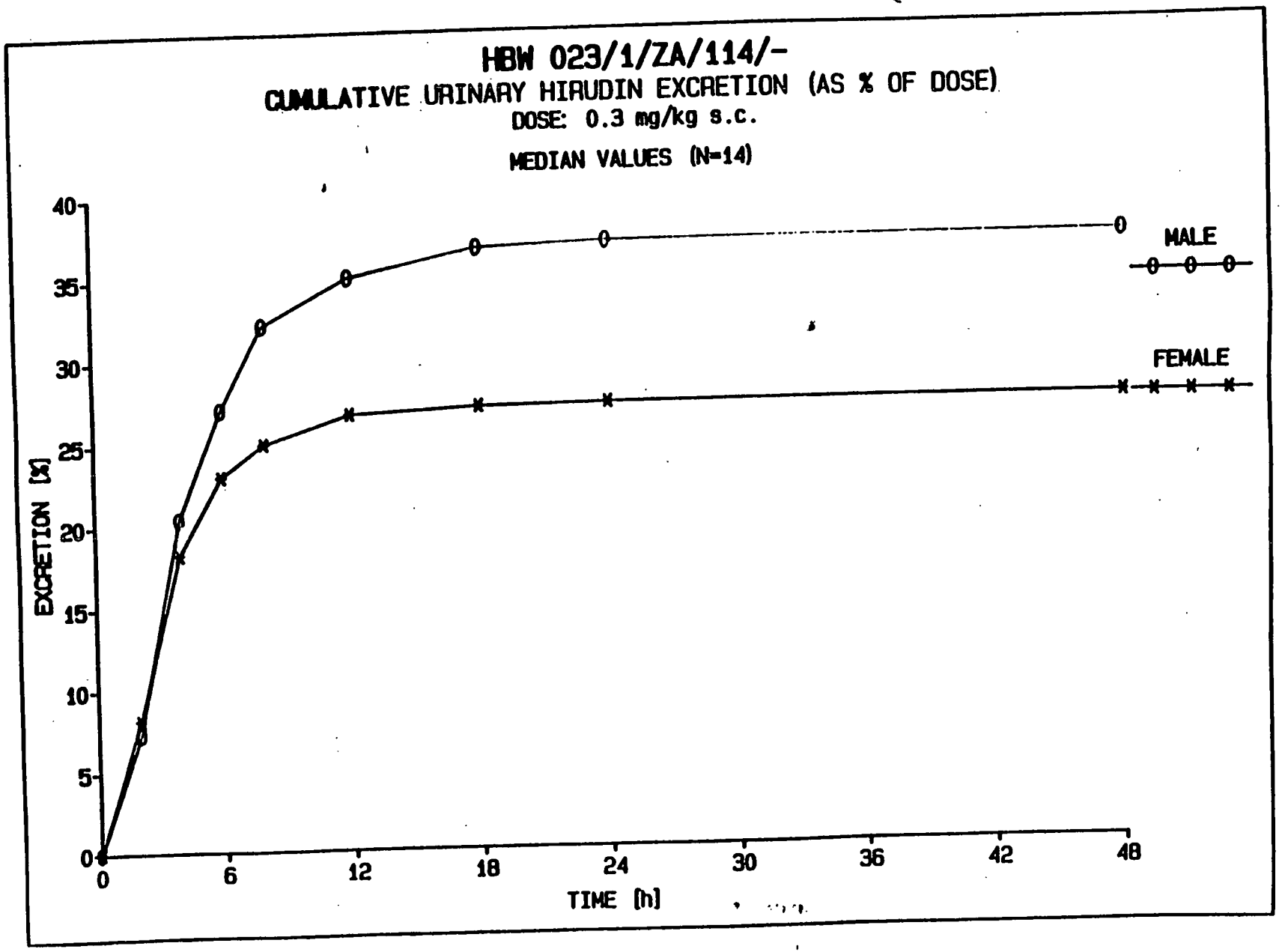

HBW 023/1/ZA/114/-
CUMULATIVE URINARY HIRUDIN EXCRETION (AS % OF DOSE)
DOSE: 0.3 mg/kg s.c.
MEDIAN VALUES (N=14)
MALE
FEMALE
EXCRETION [%]
TIME [h]
0
5
10
15
20
25
30
35
40
0
6
12
18
24
30
36
42
48

Figure 3

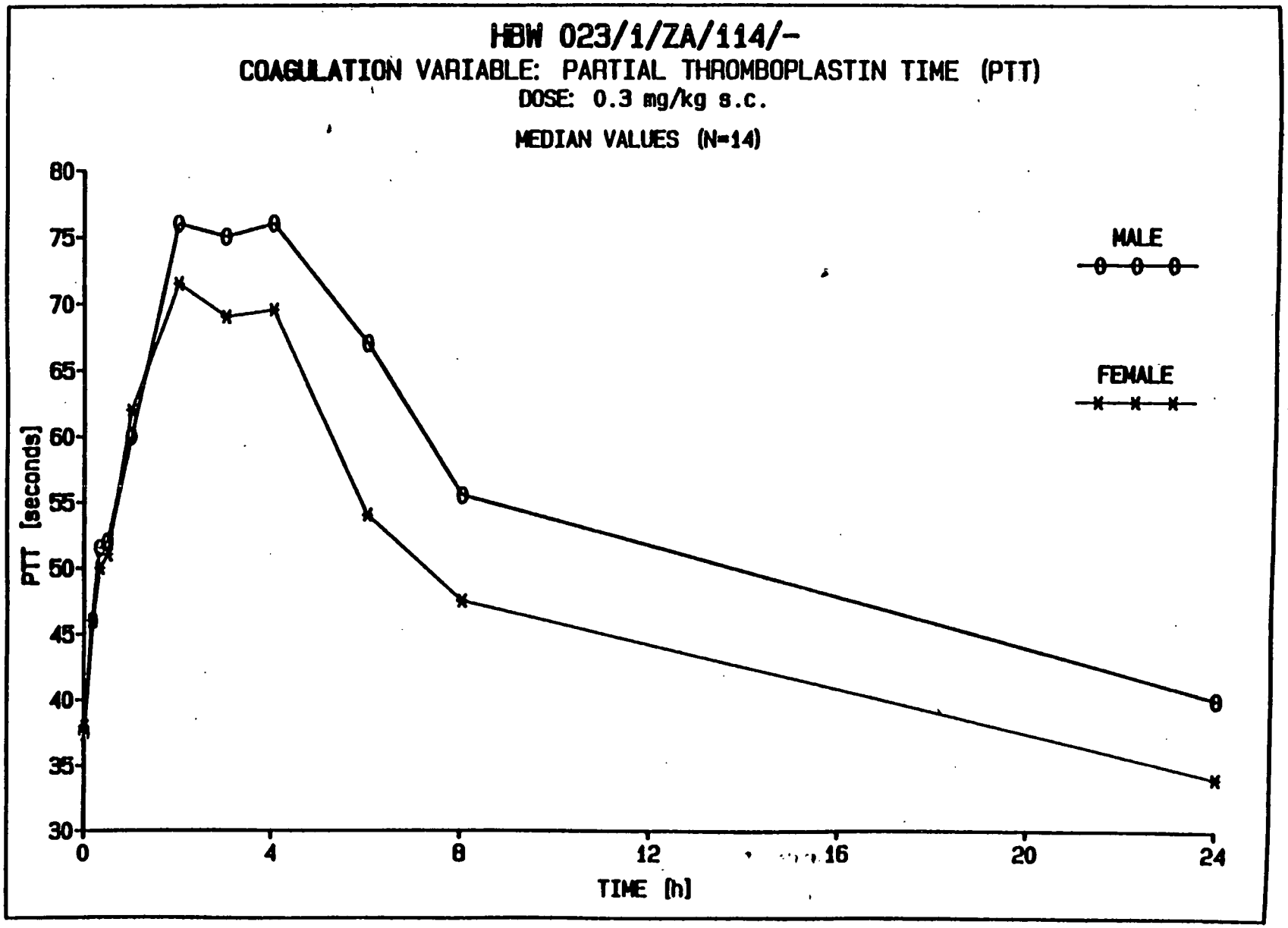

HBW 023/1/ZA/114/-
COAGULATION VARIABLE: PARTIAL THROMBOPLASTIN TIME (PTT)
DOSE: 0.3 mg/kg s.c.
MEDIAN VALUES (N=14)
MALE
FEMALE
PTT [seconds]
TIME [h]
80
75
70
65
60
55
50
45
40
35
30
0
4
8
12
16
20
24

Figure 4

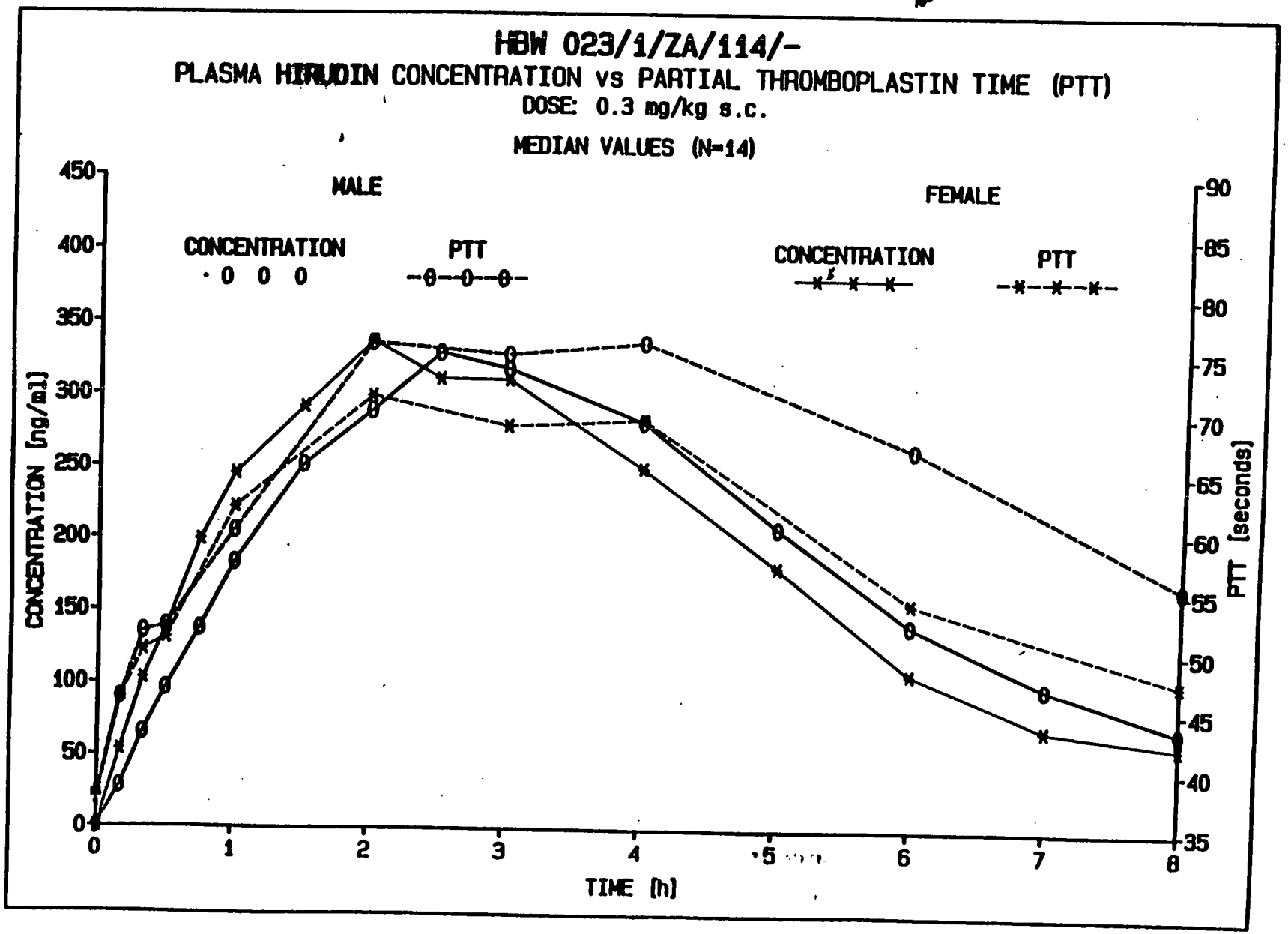

HBW 023/1/ZA/114/-
PLASMA HIRUDIN CONCENTRATION vs PARTIAL THROMBOPLASTIN TIME (PTT)
DOSE: 0.3 mg/kg s.c.
MEDIAN VALUES (N=14)
MALE
FEMALE
CONCENTRATION
PTT
CONCENTRATION
PTT
CONCENTRATION [ng/ml]
PTT [seconds]
TIME [h]
0
50
100
150
200
250
300
350
400
450
35
40
45
50
55
60
65
70
75
80
85
90
0
1
2
3
4
5
6
7
8

Figure 5

Tolerability, pharmacodynamics and pharmacokinetics of intravenously (bolus) administered hirudin (0.1 mg/kg) in the elderly

Study: A12

Investigator and Site:

Hoechst Clinic, Dept. Of Pharmacology,
University of the Orange Free State, Bloemfontein, S. Africa
Study Dates: 10 -1990
Analytical Dates: 11 - 1990

Objectives:
To investigate the tolerance, pharmacodynamics and pharmacokinetics of intravenously administered hirudin (0.1 mg/kg) in the elderly.

Formulation:
r-hirudin lyophilized powder- 10 mg powder per vial.

Study Design:
This was an open study with hirudin given intravenously (bolus) to 5 healthy males and 5 healthy females; The demographic information did not include racial breakdown of the study population. Ten mg hirudin was dissolved in 1 ml of sterile water. The required dose (0.1 mg/kg) was then removed from this solution and diluted to 10 ml with saline and injected IV into a forearm vein.

Specimens:
Blood for r-hirudin concentration: Two ml were collected at 0, 0.16,0.33, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hours after drug administration.

Blood for TT and aPTT profile: Coagulation profiles were measured before and at: 0.16, 0.33, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours.

Urine: Samples were collected and volumes were recorded at 0 and at 0-2, 2-4, 4-6 and 6-8, 8-12, 12-18, 18-24 and 24-48 hours after drug administration.

Assay:
Thrombin inhibition assay. Data on quality control samples were missing.

Result:

The following table shows the creatinine clearance (ml/min) for each subject participated in the study.

Subject #	Gender	Creatinine Clearance (ml/min)
1	F	
2	F	
3	M	
4	M	
5	M	
6	M	
7	M	
8	F	
9	F	
10	F	

The following table compares the pharmacokinetic parameters from this study to study A3 at the same dose.

Parameters	Study A12 Mean (SD)	Study A3 Mean (SD)
Cmax (ng/ml)	668 (144)	814 (99.6)
Tmax (h)	0.17 (first time point)	0.17 (first time point)
$AUC_{0\text{-}8\,h}$ (ng.h/ml)	778 (194)	650 (118)
t1/2 terminal (h)	1.74 (0.46)	1.21 (0.46)
Cltot:5h (ml/min)	182 (33.8)	170 (46.4)
Ae(0-48h) mcg	3146 (1250)	2640 (760)
Ae(0-48h) (% of dose)	40.8 (11.0)	38.1 (9.55)
Clr (ml/min)	61.8 (22.1)	68.8 (25.6)

Figure 1 shows the mean plasma hirudin concentration profile. Figure 2 shows the mean cumulative urinary hirudin excretion (% of dose). Figure 3 shows the correlation between creatinine clearance and CLtot. Figure 4 illustrate a strategy for dose adjustment in elderly population. Figure 5 shows relation between hirudin plasma levels and change in aPTT (from baseline). Figure 6 shows a cross study comparison of plasma hirudin concentration profile at

0.1 mg/kg dose (studies included A1, A5, A6 and A12). Studies A1, A5 and A6 were considered of secondary importance by this reviewer.

The following table compares the pharmacodynamic parameters (aPTT, sec) from this study to study A3 at the same dose.

Sampling time (h)	Study A12 Mean (SD)	Study A3 Mean (SD)
0	33.1 (4.05)	30.6 (7.11)
0.17	76.4 (10.5)	77.1 (11.0)
1	55.3 (6.26)	51.6 (12.4)
2	49.8 (6.81)	42.3 (11.1)
4	43.6 (4.67)	NA
5	NA	35.0 (12.1)
8	38.1 (3.51)	NA

Comment: The population PK analysis has shown that Cl_{tot} decreases with age. However, this decrease is due to inverse relation of CrCl with age.

Conclusion:

Age does not appear to be a clinically important covariate from dosage modification prospective. Tolerability and pharmacodynamic response at 0.1 mg/kg dose were similar in young and elderly population. Any dose adjustment for elderly should be based on creatinine clearance. Dosage adjustment assumes that there is no change in non-renal clearance. Pharmacokinetics and dynamics did not differ between males and females.

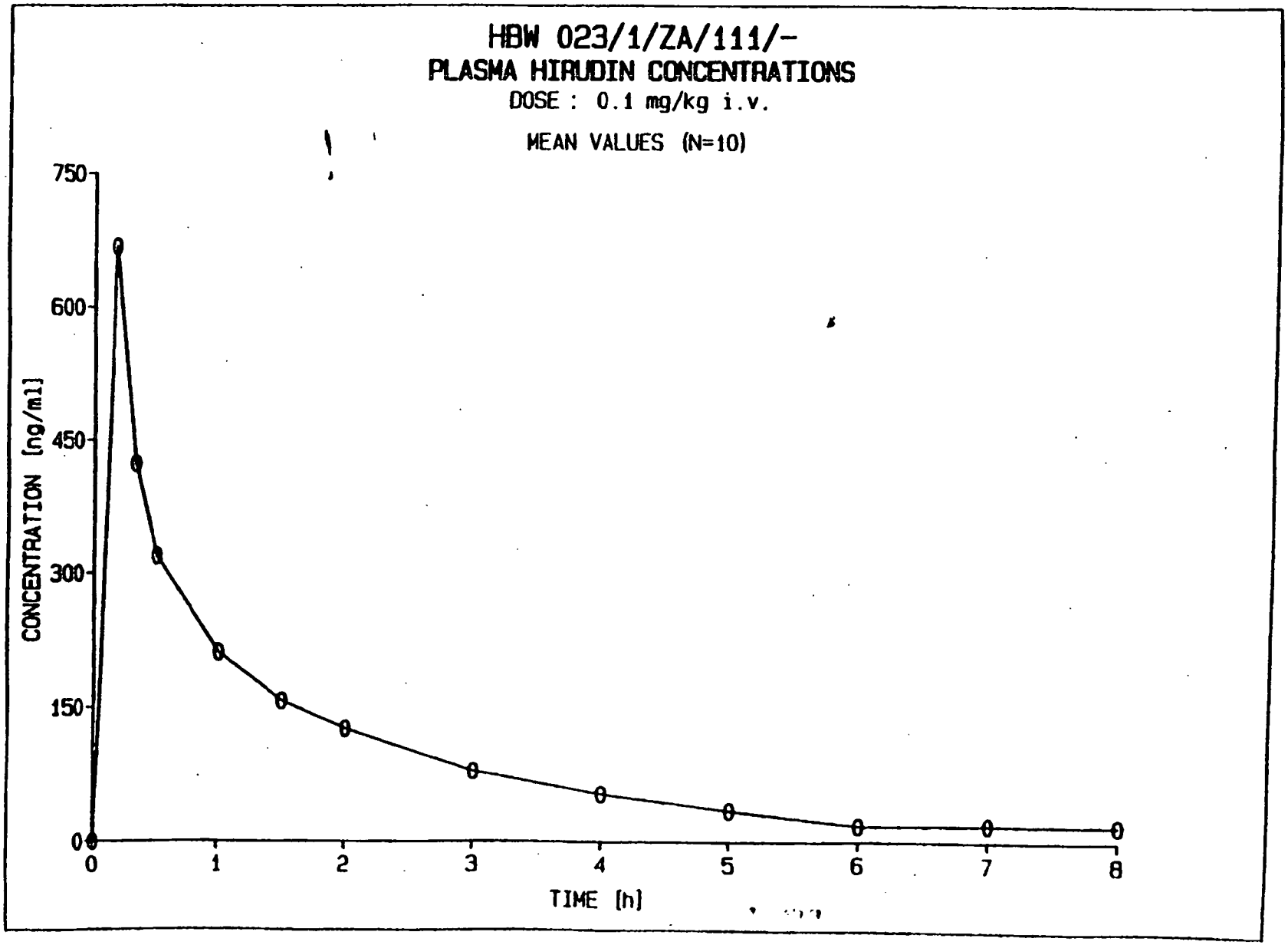

HBW 023/1/ZA/111/-
PLASMA HIRUDIN CONCENTRATIONS
DOSE : 0.1 mg/kg i.v.
MEAN VALUES (N=10)
CONCENTRATION [ng/ml]
750
600
450
300
150
0
0
1
2
3
4
5
6
7
8
TIME [h]

Figure 1

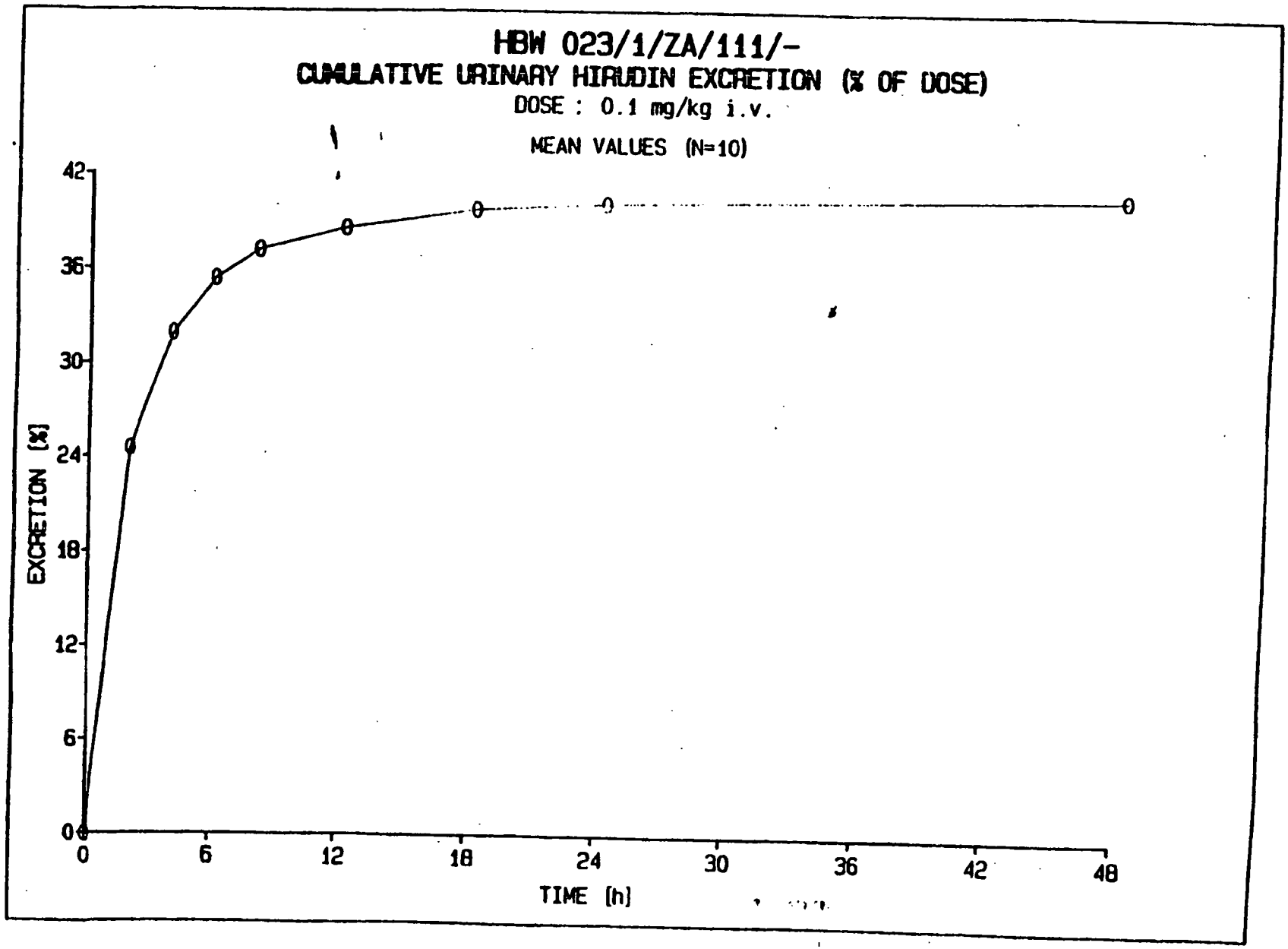

HBW 023/1/ZA/111/-
CUMULATIVE URINARY HIRUDIN EXCRETION (% OF DOSE)
DOSE : 0.1 mg/kg i.v.
MEAN VALUES (N=10)
EXCRETION [%]
0
6
12
18
24
30
36
42
TIME [h]
0
6
12
18
24
30
36
42
48

Figure # 2

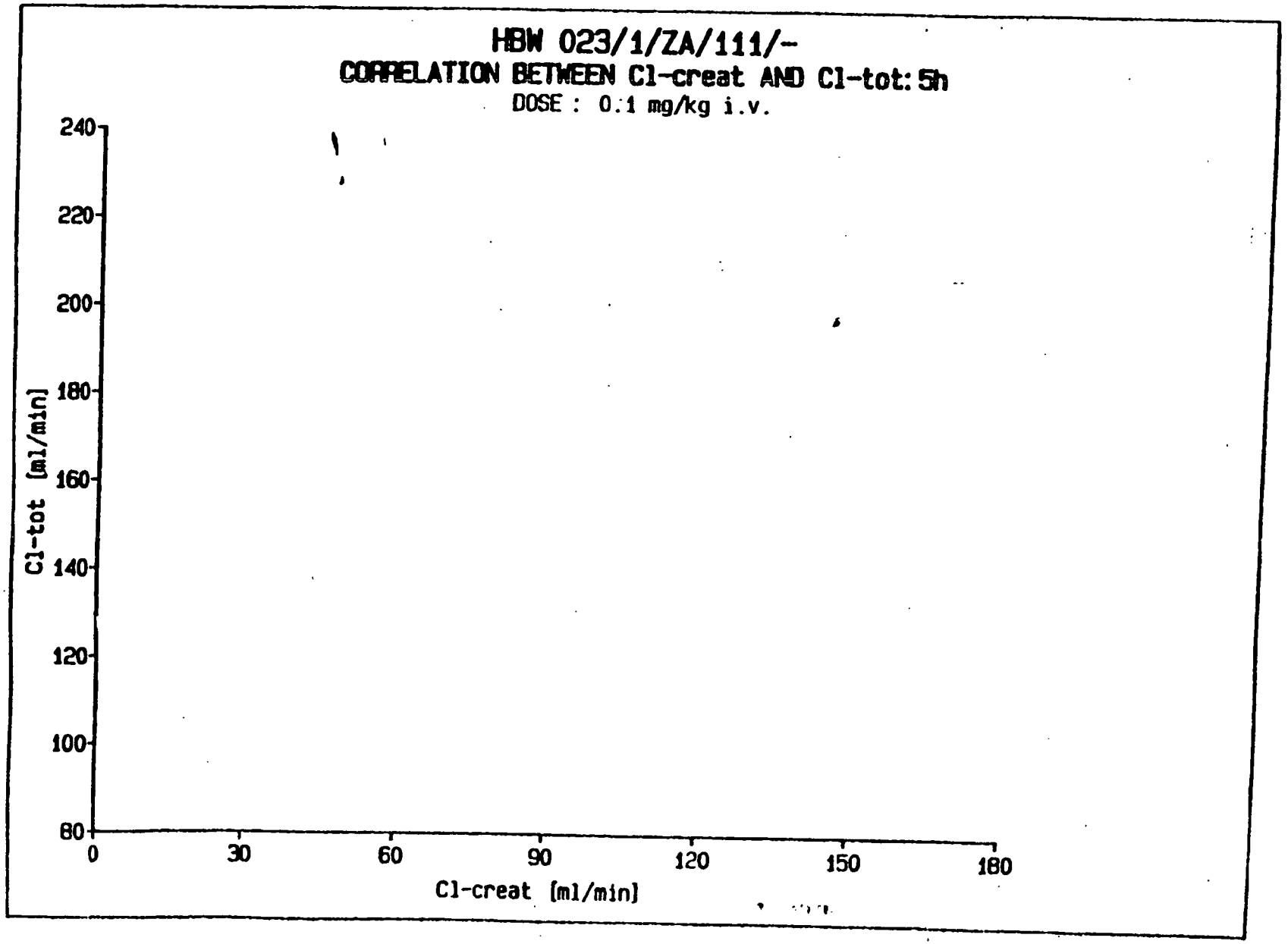

HBW 023/1/ZA/111/-
CORRELATION BETWEEN Cl-creat AND Cl-tot: 5h
DOSE : 0.1 mg/kg i.v.
Cl-tot [ml/min]
240
220
200
180
160
140
120
100
80
0
30
60
90
120
150
180
Cl-creat [ml/min]

Figure # 3

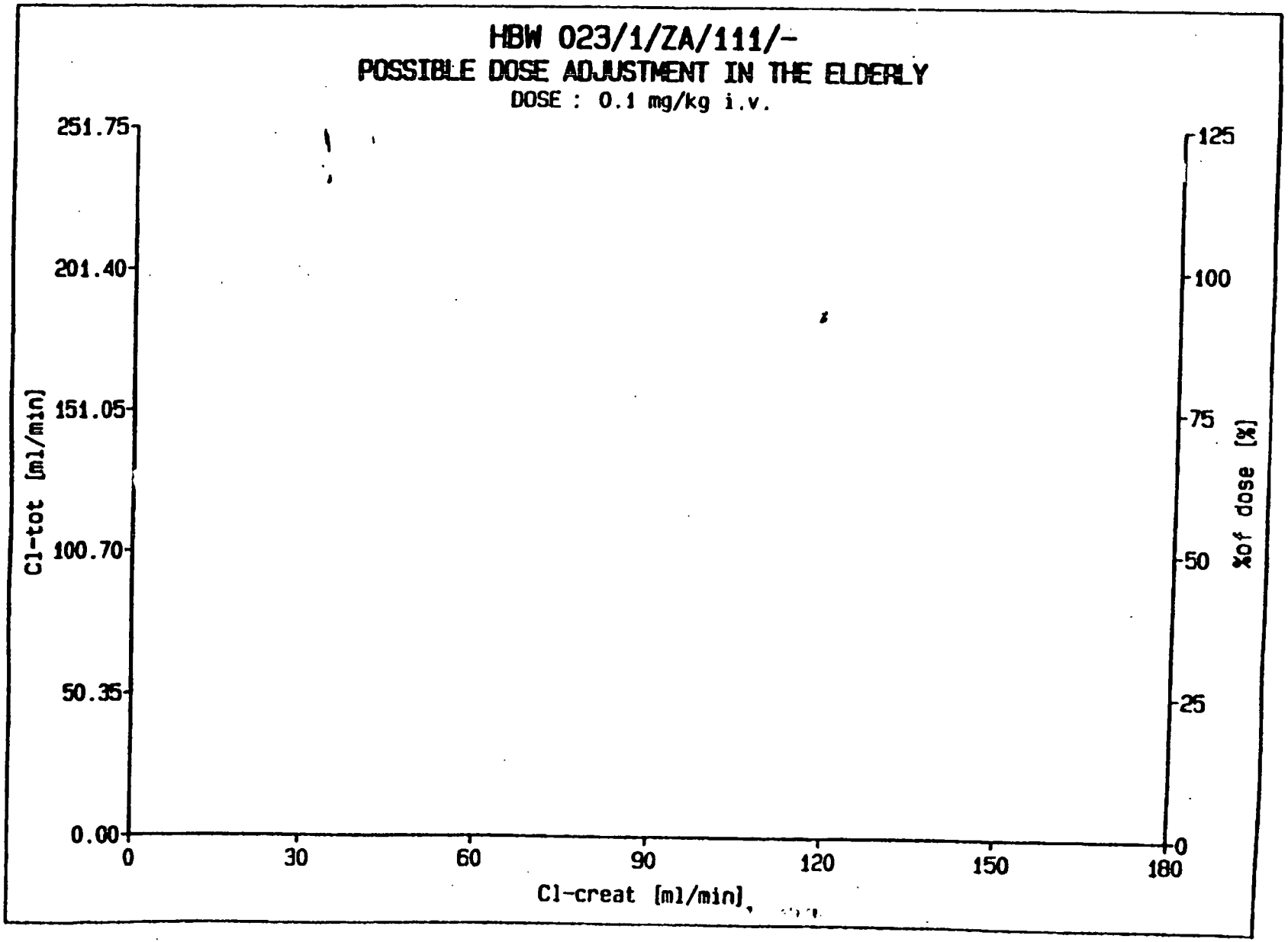

HBW 023/1/ZA/111/-
POSSIBLE DOSE ADJUSTMENT IN THE ELDERLY
DOSE : 0.1 mg/kg i.v.
Cl-tot [ml/min]
251.75
201.40
151.05
100.70
50.35
0.00
%of dose [%]
125
100
75
50
25
0
0
30
60
90
120
150
180
Cl-creat [ml/min]

Figure # 4

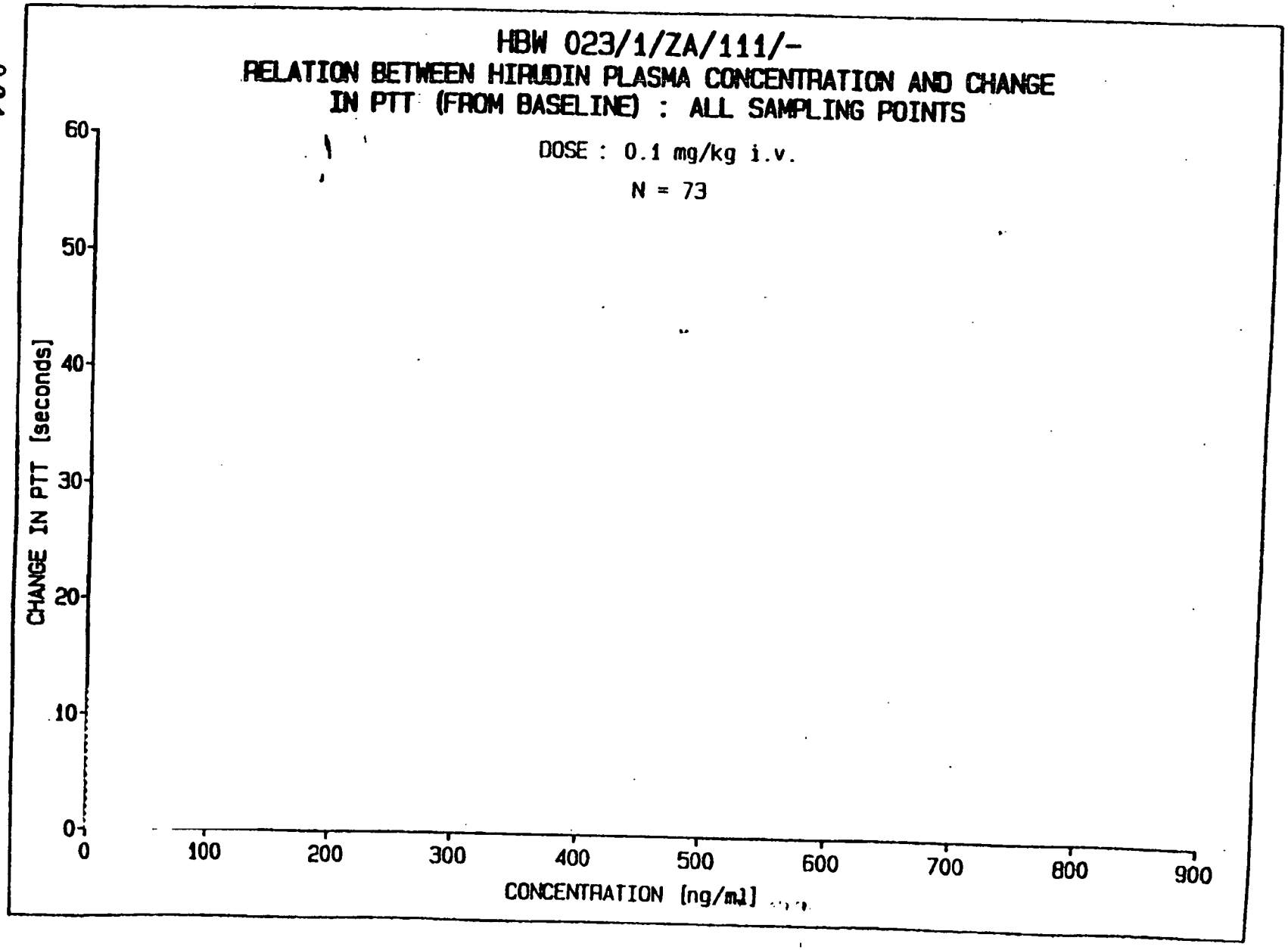

HBW 023/1/ZA/111/-
RELATION BETWEEN HIRUDIN PLASMA CONCENTRATION AND CHANGE
IN PTT (FROM BASELINE) : ALL SAMPLING POINTS
DOSE : 0.1 mg/kg i.v.
N = 73
CHANGE IN PTT [seconds]
0
10
20
30
40
50
60
0
100
200
300
400
500
600
700
800
900
CONCENTRATION [ng/ml]

Figure 45

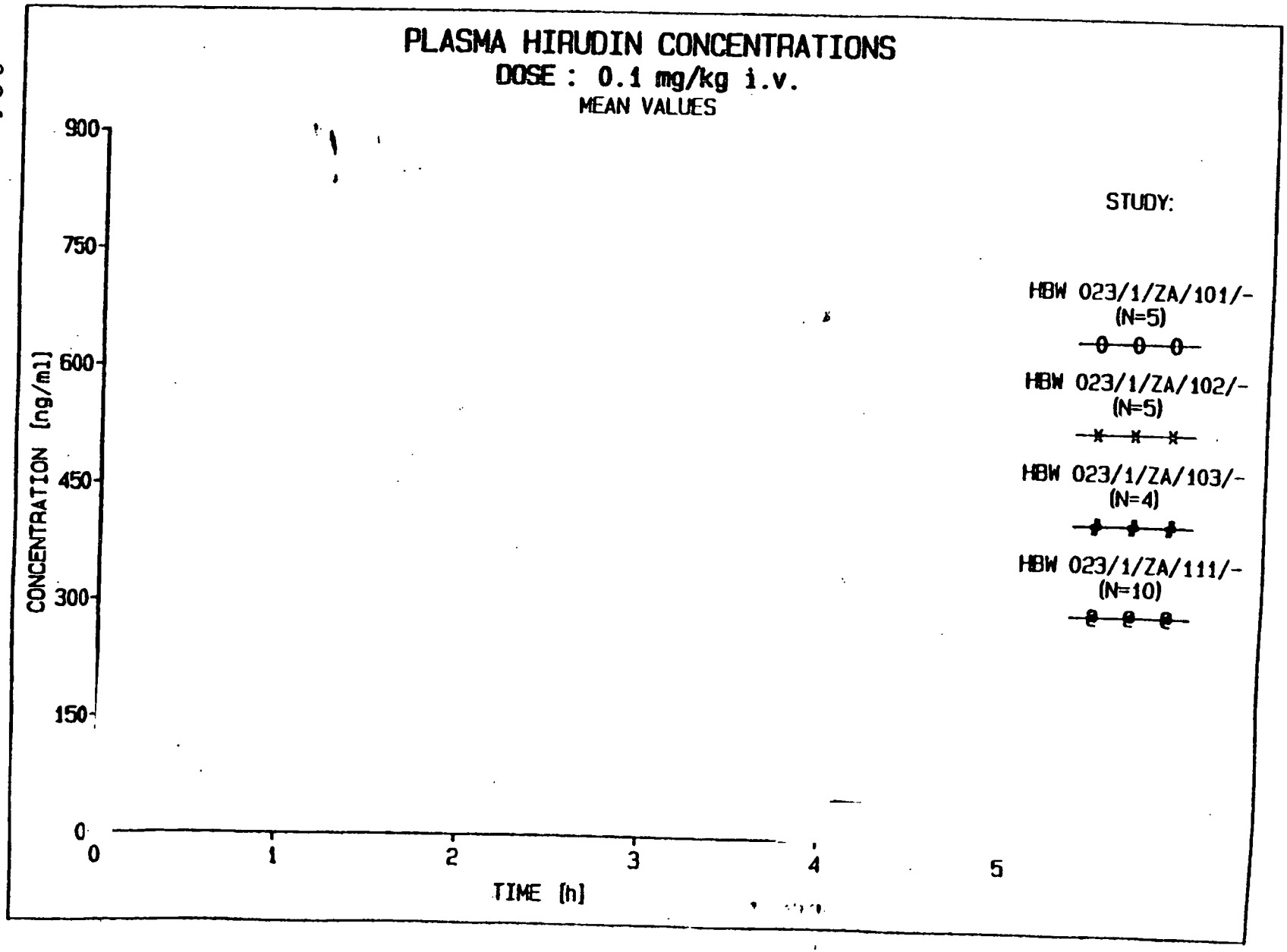

PLASMA HIRUDIN CONCENTRATIONS
DOSE : 0.1 mg/kg i.v.
MEAN VALUES
CONCENTRATION [ng/ml]
900
750
600
450
300
150
0
0
1
2
3
4
5
TIME [h]
STUDY:
HBW 023/1/ZA/101/-
(N=5)
HBW 023/1/ZA/102/-
(N=5)
HBW 023/1/ZA/103/-
(N=4)
HBW 023/1/ZA/111/-
(N=10)

Figure 6

Pharmacokinetics and pharmacodynamics of hirudin in patients with different degrees of renal impairment

Study A14

Investigator and Site:

Study Dates: August 1992 - August 1994
Analytical Dates: May 1993 - March 1995

Objectives:
Pharmacokinetics and pharmacodynamics of a single intravenous dose of r-hirudin in patients with varying degrees of kidney function

Formulation:
r-hirudin, 10 mg lyophilisate, single-dose, 0.05 mg/kg given as an IV infusion over 60 minutes, batch 12 (Gent), batch 19 (other centers)

Study Design:
This was a open, single dose study, where 0.05 mg/kg dose was administered as an IV infusion over 60 minutes to 17 male or female patients,
The patients were selected on the basis of their creatinine clearance at screening as follows

Group I:	ml/min (N=2)
Group II:	ml/min (N= 5)
Group III:	ml/min (N= 5)
Group IV:	< 10 ml/min (N = 5)

Specimen:
For determination of hirudin concentrations venous blood (3.6 ml) was collected into a tube containing 0.4 ml 3.8 % sodium citrate. These samples were taken before medication (time 0:00 h:min) and 0:30, 1:00 (end of infusion), 1:15, 1:30, 2:00, 3:00, 5:00, 7:00, 9:00, 24:00, 48:00, 72:00, 96:00 and 120:00 h:min thereafter. The samples were immediately centrifuged and the plasma (at least 1 ml, sample before medication 5 ml) stored frozen (-20 °C) until analysis.

For TT and aPTT determination the samples were collected into commercially available citrated tubes; the coagulation tests were performed immediately. Blood samples (2 ml) were collected at the following times: before medication and 1 (end of infusion), 2, 3, 5, 7, 9, 24, 48, 72, 96 and 120 hours thereafter.

For determination of creatinine concentration 1 ml of blood was collected into uncoated tubes at the following times: before medication, 2, 5, 9, 24, 48, 72, 96 and 120 hours after start of infusion.

Blood samples (hirudin, aPTT, TT, creatinine) at 72 and 96 hours were only collected in groups II - IV, at 120 hours only in groups III and IV.

The patients emptied their bladders immediately before infusion of the study drug to provide a baseline sample. During the whole study total volumes of urine were collected in the following fractions:

0 - 2, 2 - 5, 5 - 9, 9 - 24, 24 - 48, 48 - 72, 72 - 96 and 96 - 120 hours after start of infusion.

As for blood sampling, the samples later than 48 hours were only collected in groups II - IV, the sample between 96 and 120 hours only in groups III and IV.

Assay:

Hirudin concentrations in plasma and urine: Antibodies against hirudin and yeast:

Results:

The following table shows the mean values and ranges of the pharmacokinetic variables.

Plasma:

Variable	Group I (n = 3)	Group II (n = 5)	Group III (n = 5)	Group IV (n = 3)
C_{max} [1] (ng/ml)	260	315	404	340
AUDC (ng.h/ml)	433	1028	3214	12737
$t_{1/2;b}$ (h)	1.35*	2.72	6.72	9.69*
CL_{tot} (ml/min)	168	95.9	28.8	4.74
V_{ss} (l)	11.5	16.3	23.6	22.4

1) at the end of infusion

* n = 2

** n = 4

Urine:

The mean values and ranges of the total urinary excretion calculated up to the last measuring point

per group (Ae) and average renal clearance (Clren) of r-hirudin were as follows

Variable	Group I (0 - 48h) (n = 3)	Group II (0 - 96h) (n = 5)	Group III (0 - 120h) (n = 5)	Group IV (0 - 120h) (n = 3)
Ae (% of dose)	29.2	35.2	50.6	50.6
CL_{ren} (ml/min)	45.7	32.5	15.5	2.77

Figure 1 show median plasma hirudin concentrations versus time for all patients. Figure 2a and 2b show mean and median cumulative urinary hirudin excretion (% of dose), respectively. Figure 3 show the median aPTT versus time profile for all patients. Figure 4a and 4b show superimposed plots of plasma concentration and aPTT versus time. Figure 5 illustrate the median serum creatinine concentration versus time plot. Figure 6 show the bar plot for median creatinine clearance for all four groups. Figure 7, 8 and 9 show relation between CrCl and CLtot and possible dose adjustment strategy. Figure 10 show relation between CrCl and Clren. Figure 11 illustrate the PK-PD relationship.

Pharmacodynamics:

The mean values and ranges of aPTT (seconds) at a few selected sampling times were

Sampling time (h)	Group I (n = 3)	Group II (n = 5)	Group III (n = 5)	Group IV (n = 3)
0	32.0**	30.6	37.6	36.0
1	54.7	57.2	81.2	96.3
5	32.3	38.5****	47.6	57.3
24	30.0	30.8****	65.6	48.0
72	23.0*	31.8****	33.0***	36.0**
*	n = 1		***	n = 3
**	n = 2		****	n = 4

Conclusion:

Renal function appears to be an important factor in influencing the renal and total clearance of r-hirudin. There appears to be a linear relationship between CrCl and total clearance of r-hirudin. In this study concentration-response (aPTT) relationship was established. A dose reduction, directly proportional to reduction in CrCl is recommended based on data observed in this study.

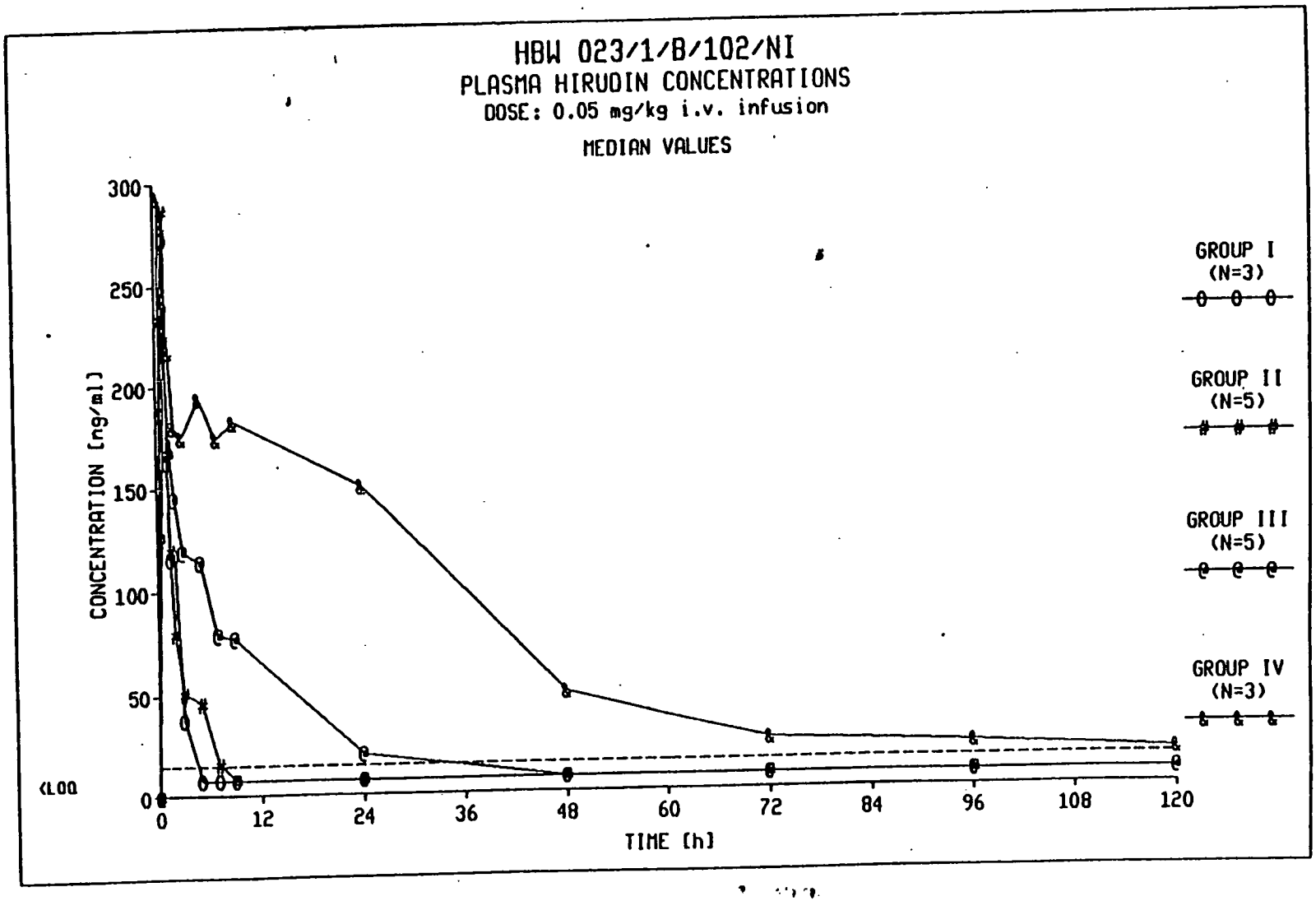

HBW 023/1/B/102/NI
PLASMA HIRUDIN CONCENTRATIONS
DOSE: 0.05 mg/kg i.v. infusion
MEDIAN VALUES
CONCENTRATION [ng/ml]
300
250
200
150
100
50
0
<LOQ
0
12
24
36
48
60
72
84
96
108
120
TIME [h]
GROUP I
(N=3)
GROUP II
(N=5)
GROUP III
(N=5)
GROUP IV
(N=3)

Figure 1

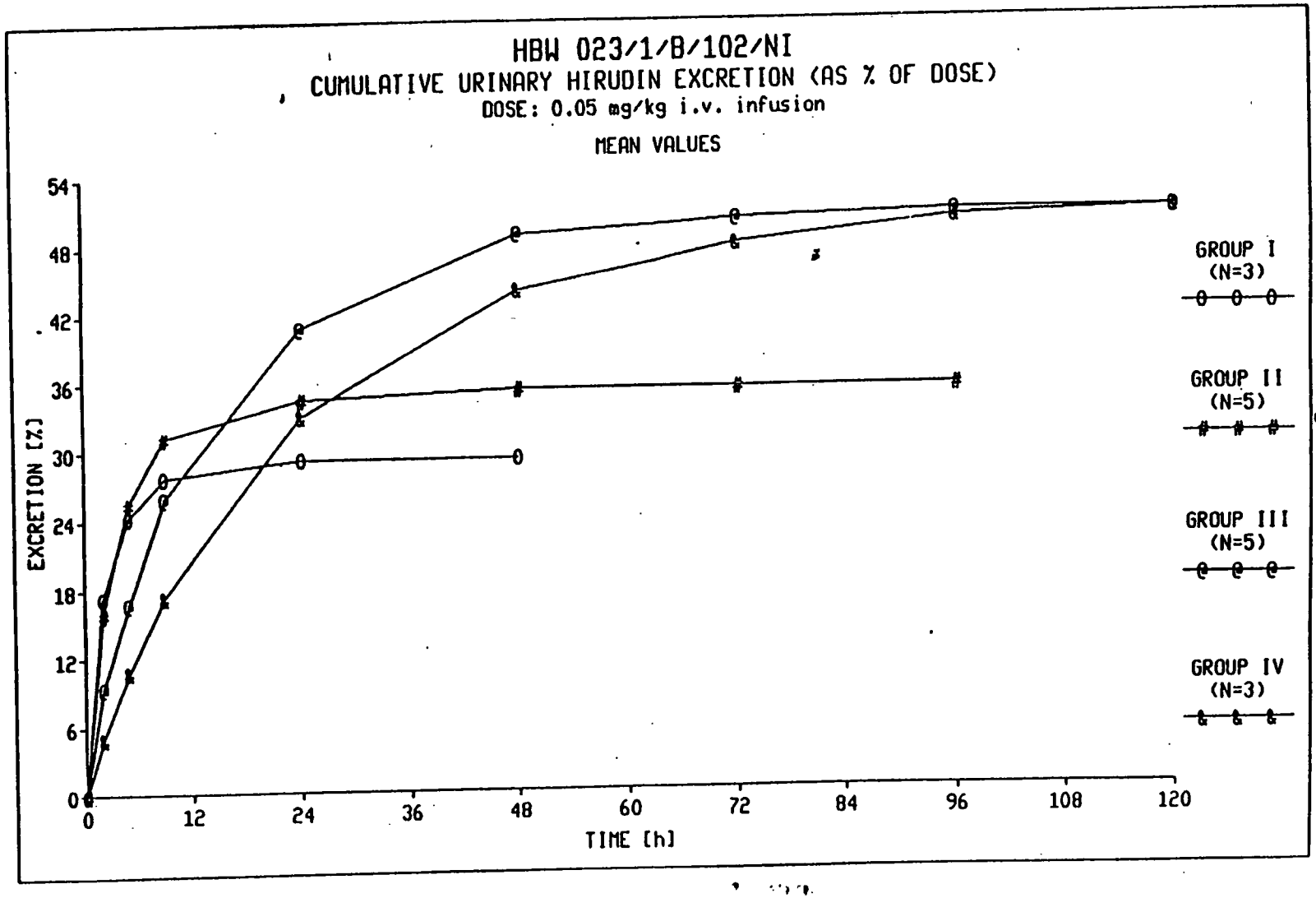

HBW 023/1/B/102/NI
CUMULATIVE URINARY HIRUDIN EXCRETION (AS % OF DOSE)
DOSE: 0.05 mg/kg i.v. infusion
MEAN VALUES
EXCRETION [%]
TIME [h]
0
6
12
18
24
30
36
42
48
54
0
12
24
36
48
60
72
84
96
108
120
GROUP I
(N=3)
GROUP II
(N=5)
GROUP III
(N=5)
GROUP IV
(N=3)

Figure 2a

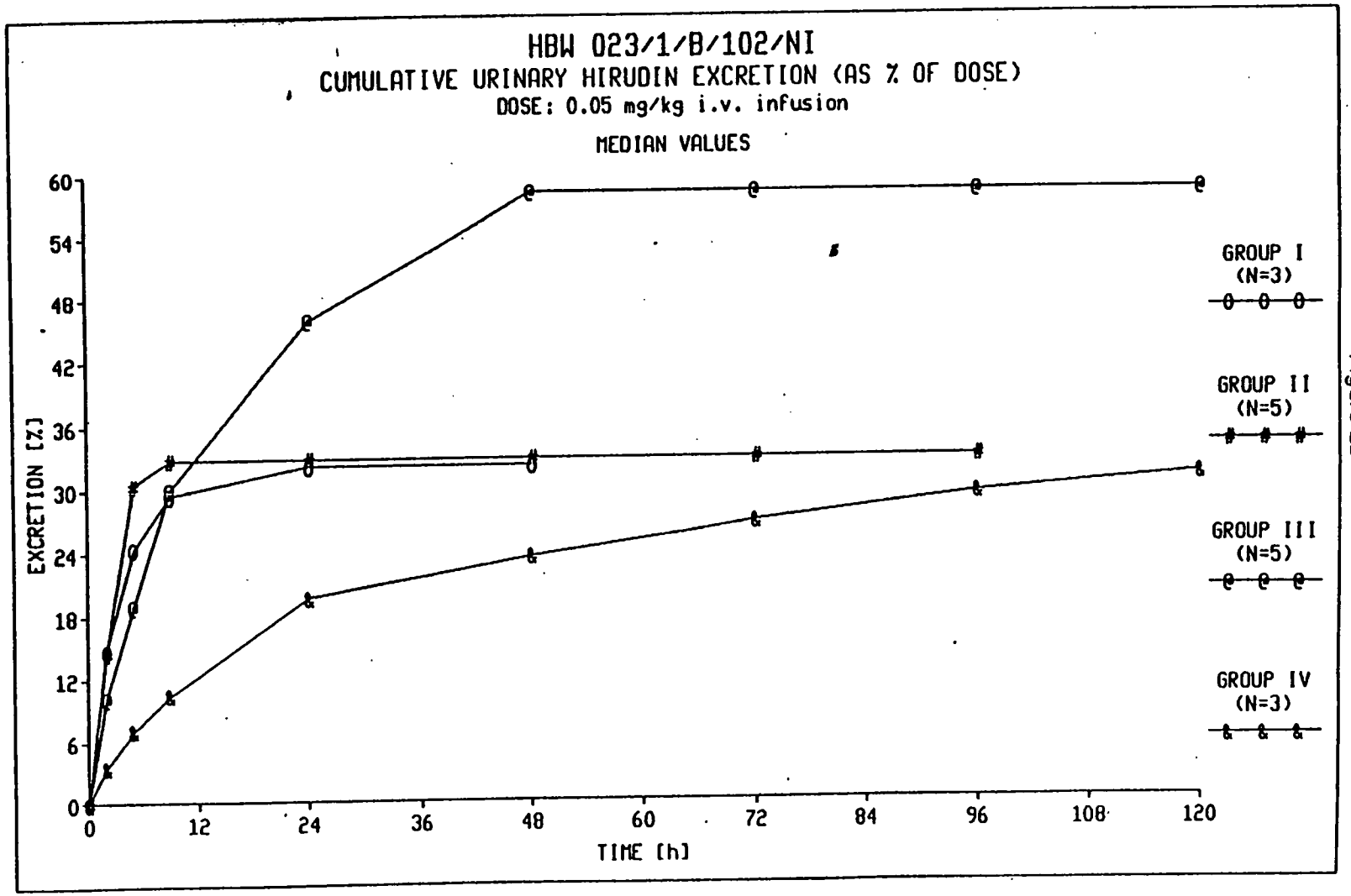

HBW 023/1/B/102/NI
CUMULATIVE URINARY HIRUDIN EXCRETION (AS % OF DOSE)
DOSE: 0.05 mg/kg i.v. infusion
MEDIAN VALUES
EXCRETION [%]
TIME [h]
0
6
12
18
24
30
36
42
48
54
60
0
12
24
36
48
60
72
84
96
108
120
GROUP I
(N=3)
GROUP II
(N=5)
GROUP III
(N=5)
GROUP IV
(N=3)

Figure 2b

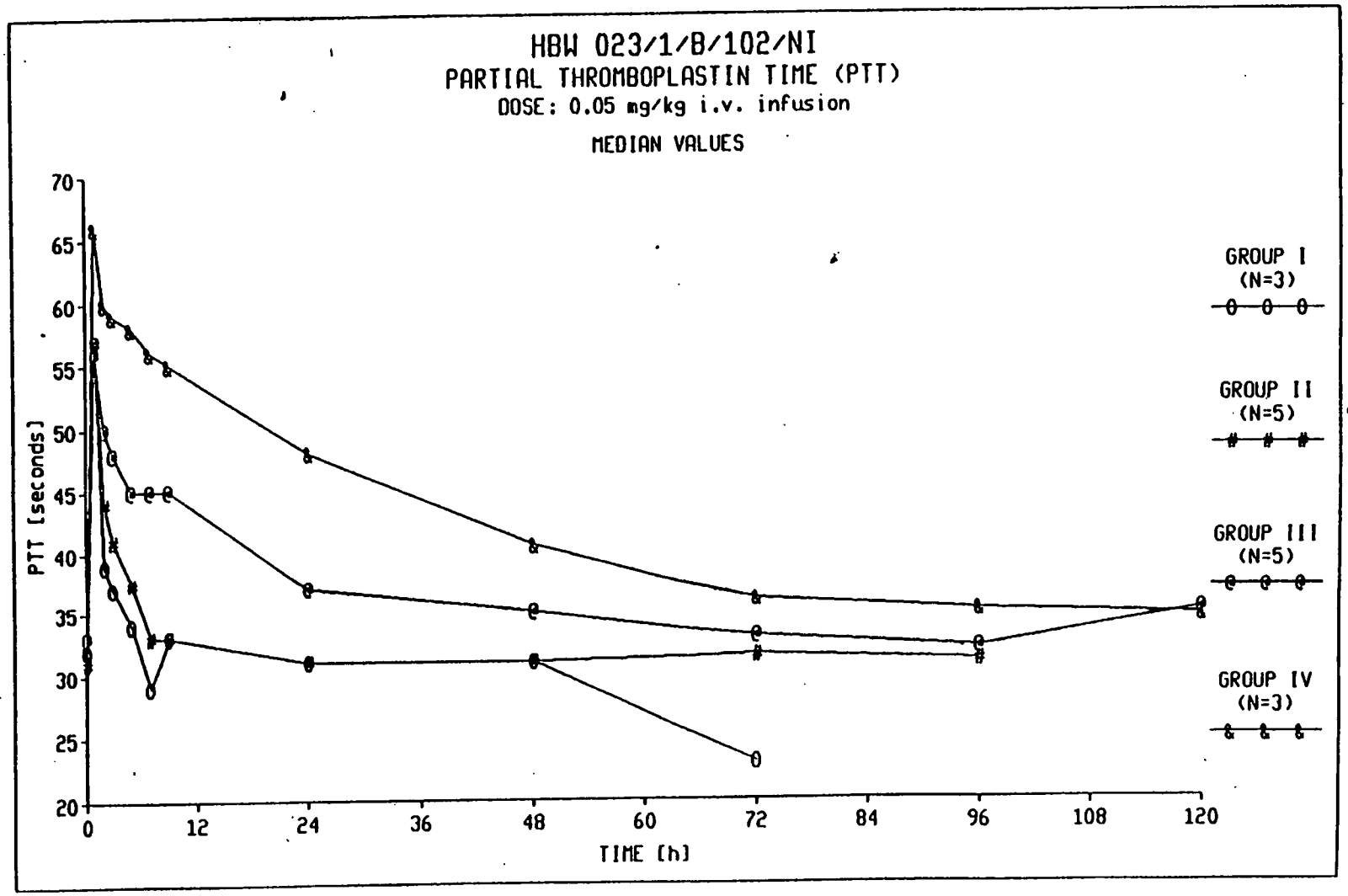
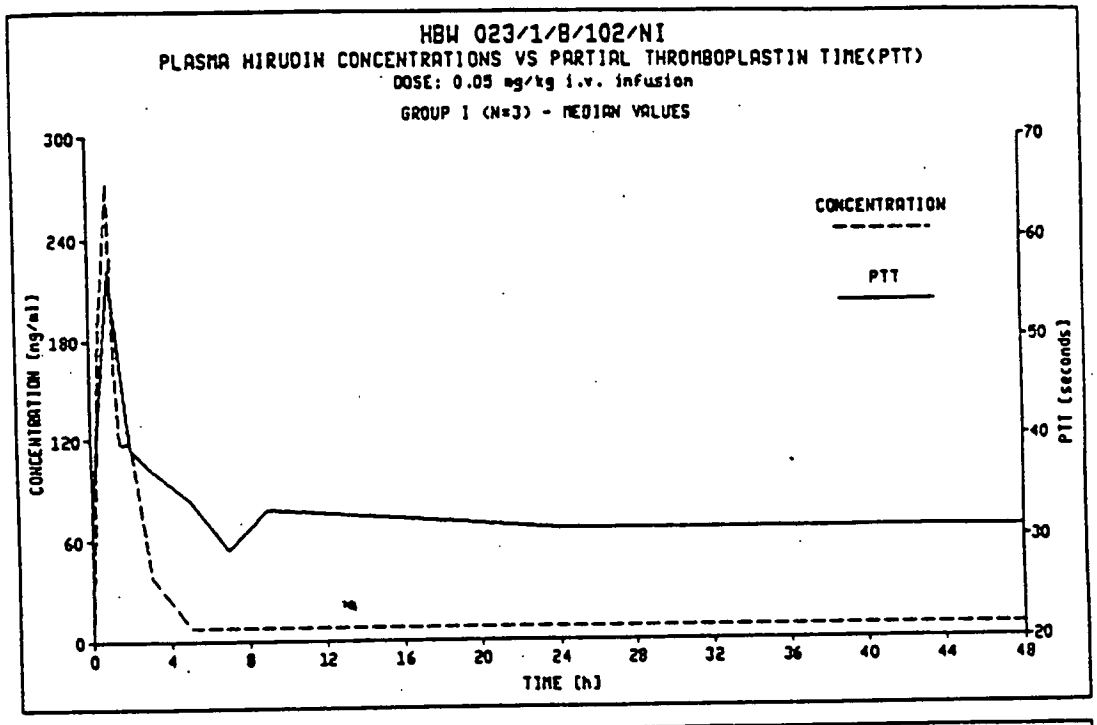

HBW 023/1/B/102/NI
PARTIAL THROMBOPLASTIN TIME (PTT)
DOSE: 0.05 mg/kg i.v. infusion
MEDIAN VALUES
PTT [seconds]
TIME [h]
70
65
60
55
50
45
40
35
30
25
20
0
12
24
36
48
60
72
84
96
108
120
GROUP I
(N=3)
GROUP II
(N=5)
GROUP III
(N=5)
GROUP IV
(N=3)

Figure 3

Figure 4a


HBW 023/1/8/102/NI
PLASMA HIRUDIN CONCENTRATIONS VS PARTIAL THROMBOPLASTIN TIME(PTT)
DOSE: 0.05 mg/kg i.v. infusion
GROUP I (N=3) - MEDIAN VALUES
CONCENTRATION
PTT
CONCENTRATION [ng/ml]
PTT [seconds]
TIME [h]
0
4
8
12
16
20
24
28
32
36
40
44
48
60
120
180
240
300
20
30
40
50
60
70

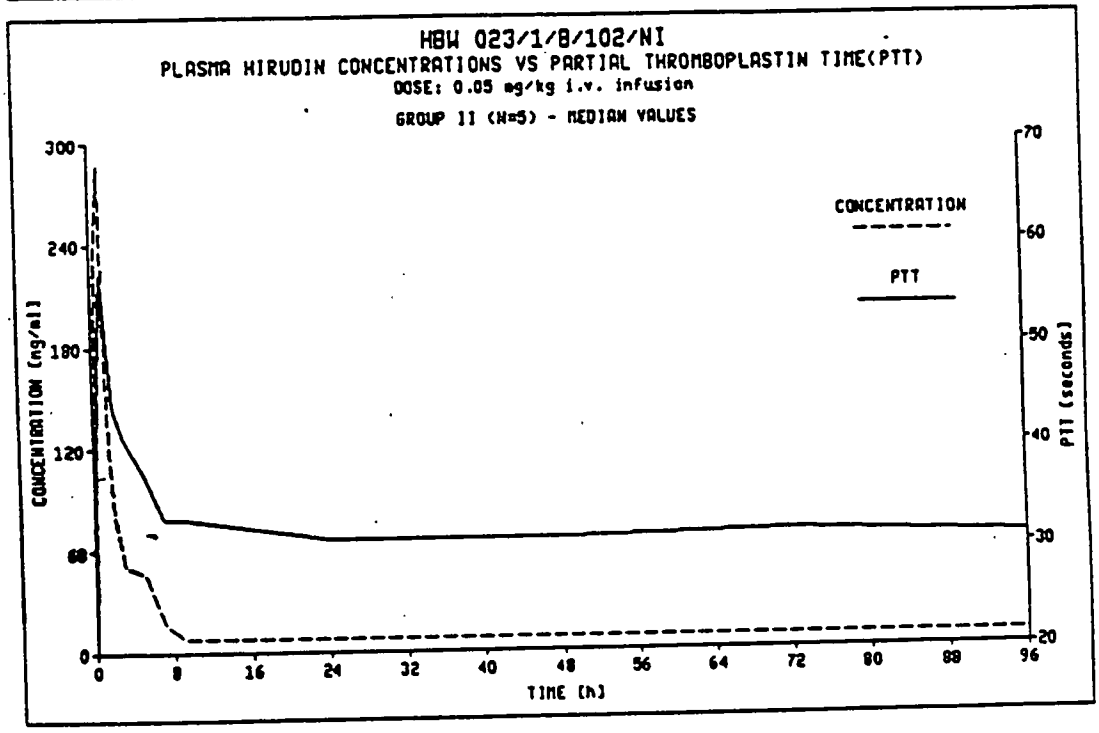
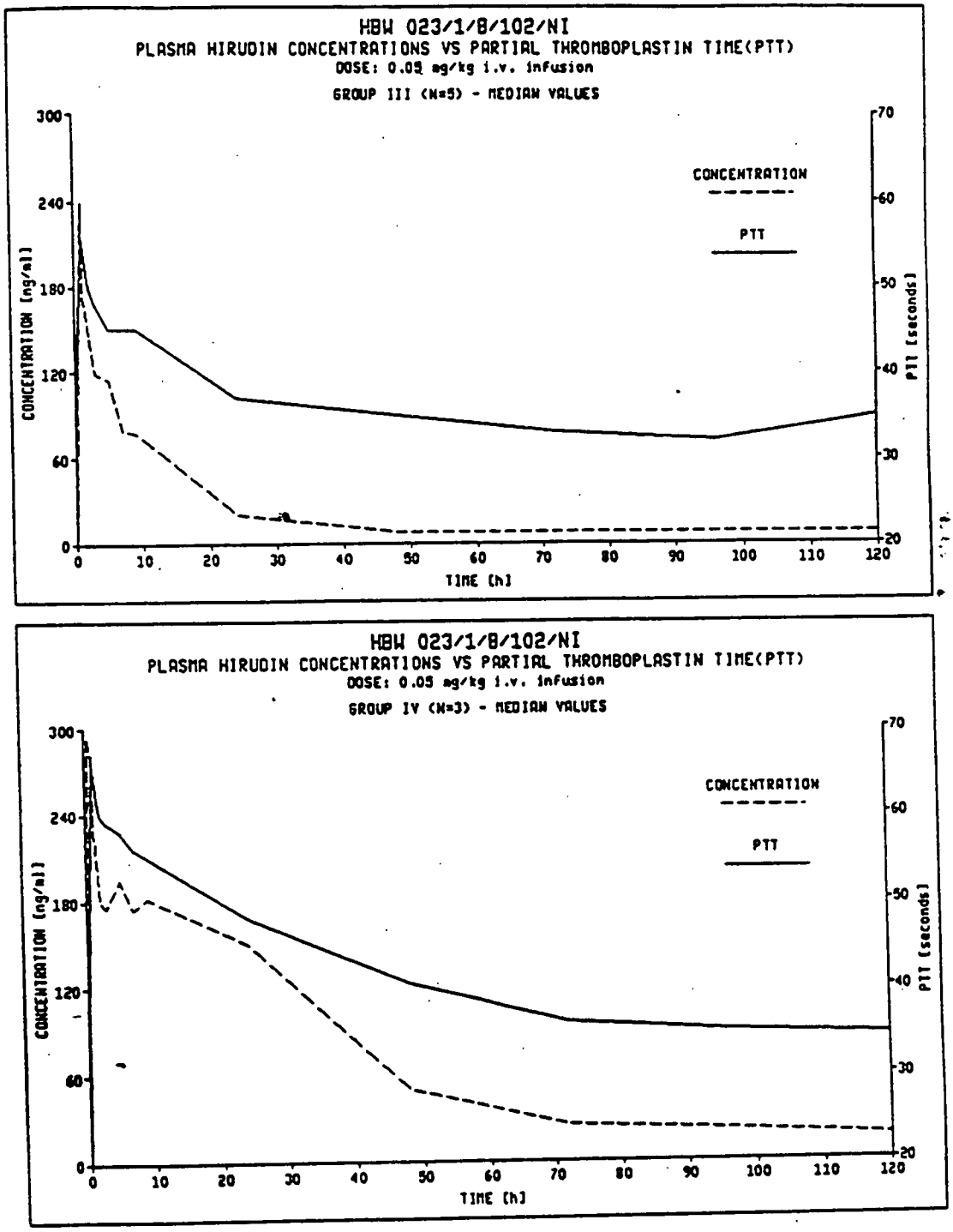

HBW 023/1/8/102/NI
PLASMA HIRUDIN CONCENTRATIONS VS PARTIAL THROMBOPLASTIN TIME(PTT)
DOSE: 0.05 mg/kg i.v. infusion
GROUP II (N=5) - MEDIAN VALUES
CONCENTRATION
PTT
CONCENTRATION [ng/ml]
PTT [seconds]
TIME [h]
0
8
16
24
32
40
48
56
64
72
80
88
96
60
120
180
240
300
20
30
40
50
60
70

Figure 4b


HBW 023/1/8/102/NI
PLASMA HIRUDIN CONCENTRATIONS VS PARTIAL THROMBOPLASTIN TIME(PTT)
DOSE: 0.05 mg/kg i.v. infusion
GROUP III (N=5) - MEDIAN VALUES
CONCENTRATION
PTT
CONCENTRATION [ng/ml]
PTT [seconds]
TIME [h]
HBW 023/1/8/102/NI
PLASMA HIRUDIN CONCENTRATIONS VS PARTIAL THROMBOPLASTIN TIME(PTT)
DOSE: 0.05 mg/kg i.v. infusion
GROUP IV (N=3) - MEDIAN VALUES
CONCENTRATION
PTT
CONCENTRATION [ng/ml]
PTT [seconds]
TIME [h]

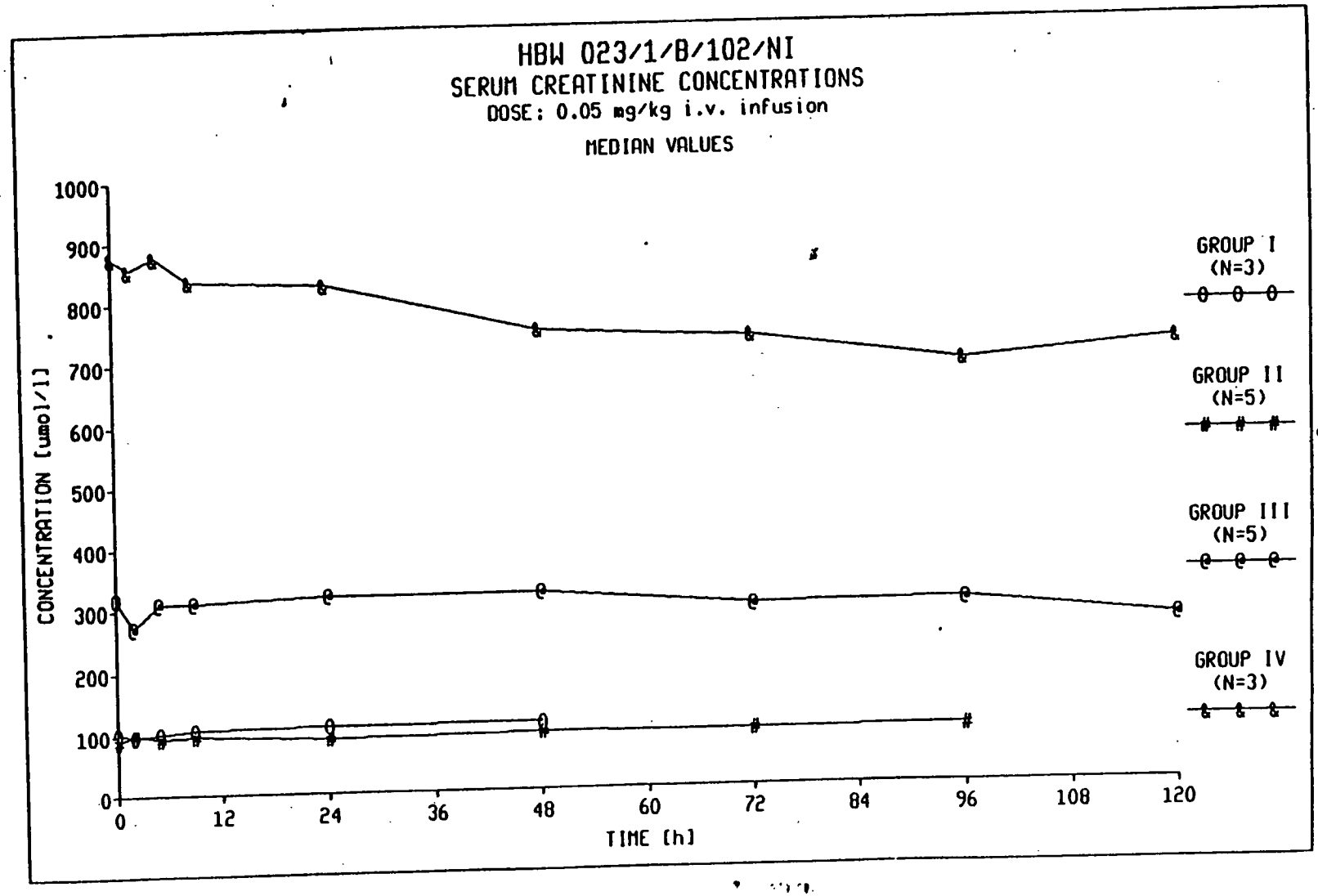

HBW 023/1/B/102/NI
SERUM CREATININE CONCENTRATIONS
DOSE: 0.05 mg/kg i.v. infusion
MEDIAN VALUES
CONCENTRATION [umol/l]
1000
900
800
700
600
500
400
300
200
100
0
0
12
24
36
48
60
72
84
96
108
120
TIME [h]
GROUP I
(N=3)
GROUP II
(N=5)
GROUP III
(N=5)
GROUP IV
(N=3)

Figure 5

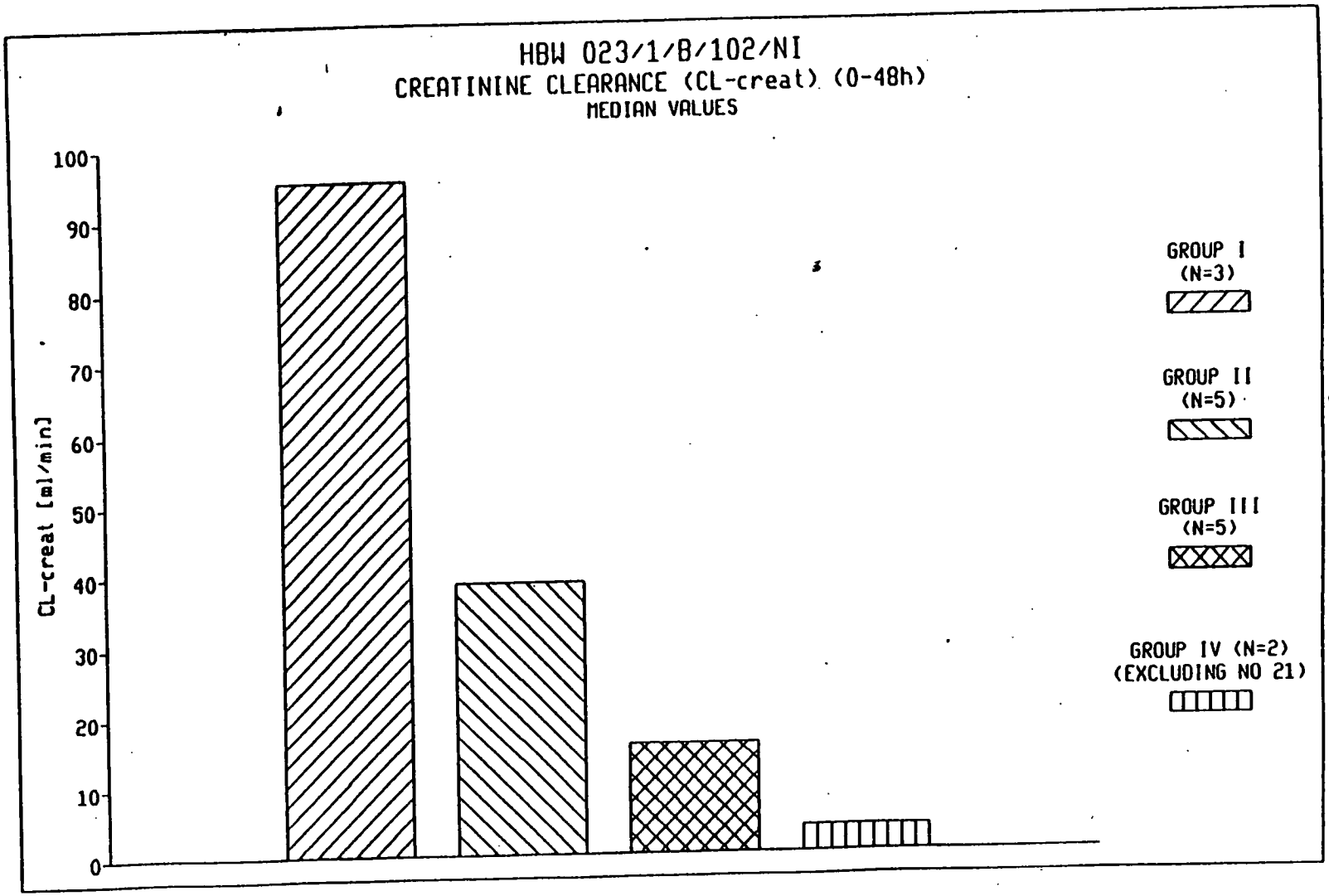

HBW 023/1/B/102/NI
CREATININE CLEARANCE (CL-creat) (0-48h)
MEDIAN VALUES
CL-creat [ml/min]
100
90
80
70
60
50
40
30
20
10
0
GROUP I
(N=3)
GROUP II
(N=5)
GROUP III
(N=5)
GROUP IV (N=2)
(EXCLUDING NO 21)

Figure 6

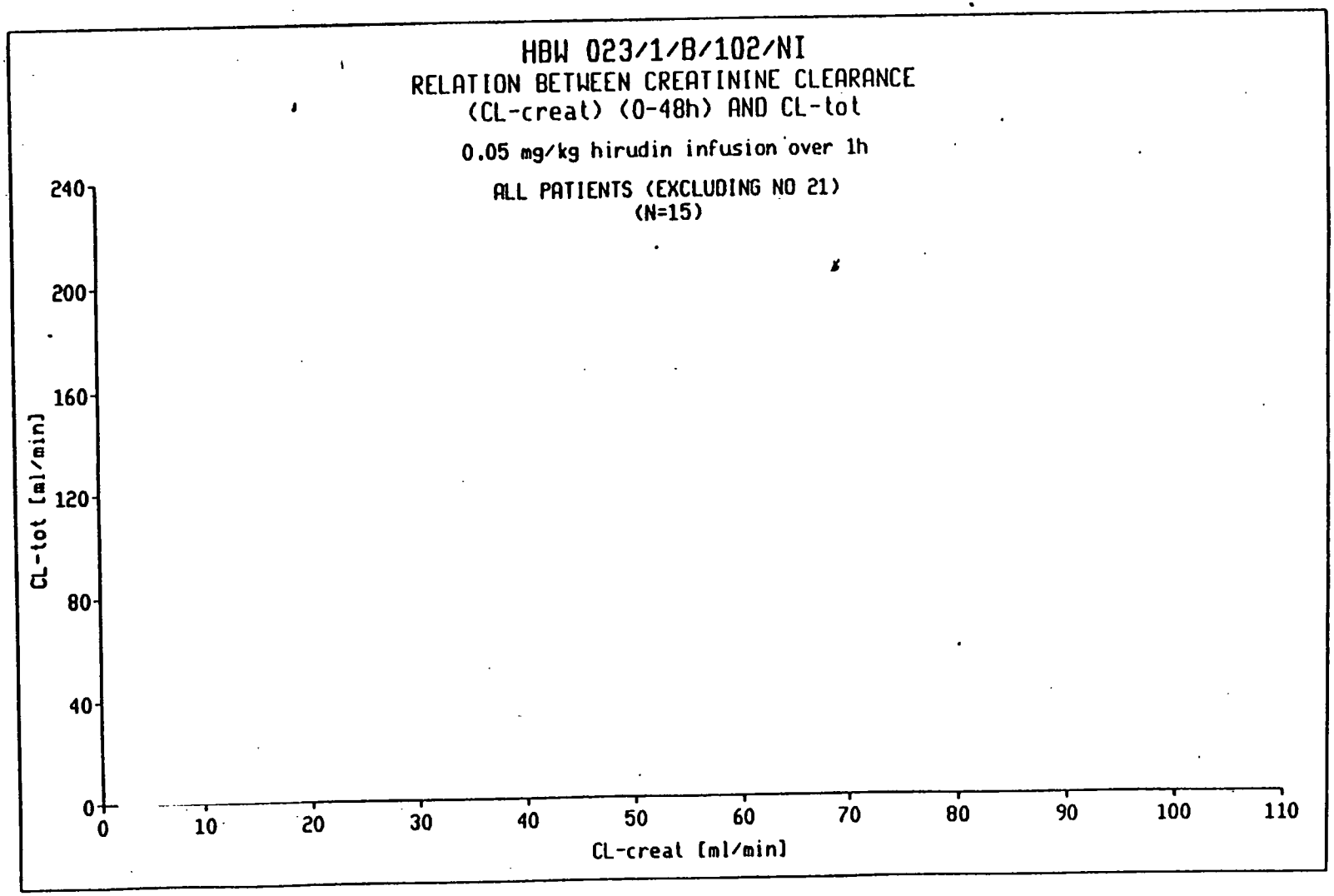

HBW 023/1/B/102/NI
RELATION BETWEEN CREATININE CLEARANCE
(CL-creat) (0-48h) AND CL-tot
0.05 mg/kg hirudin infusion over 1h
ALL PATIENTS (EXCLUDING NO 21)
(N=15)
CL-tot [ml/min]
240
200
160
120
80
40
0
0
10
20
30
40
50
60
70
80
90
100
110
CL-creat [ml/min]

Figure 7

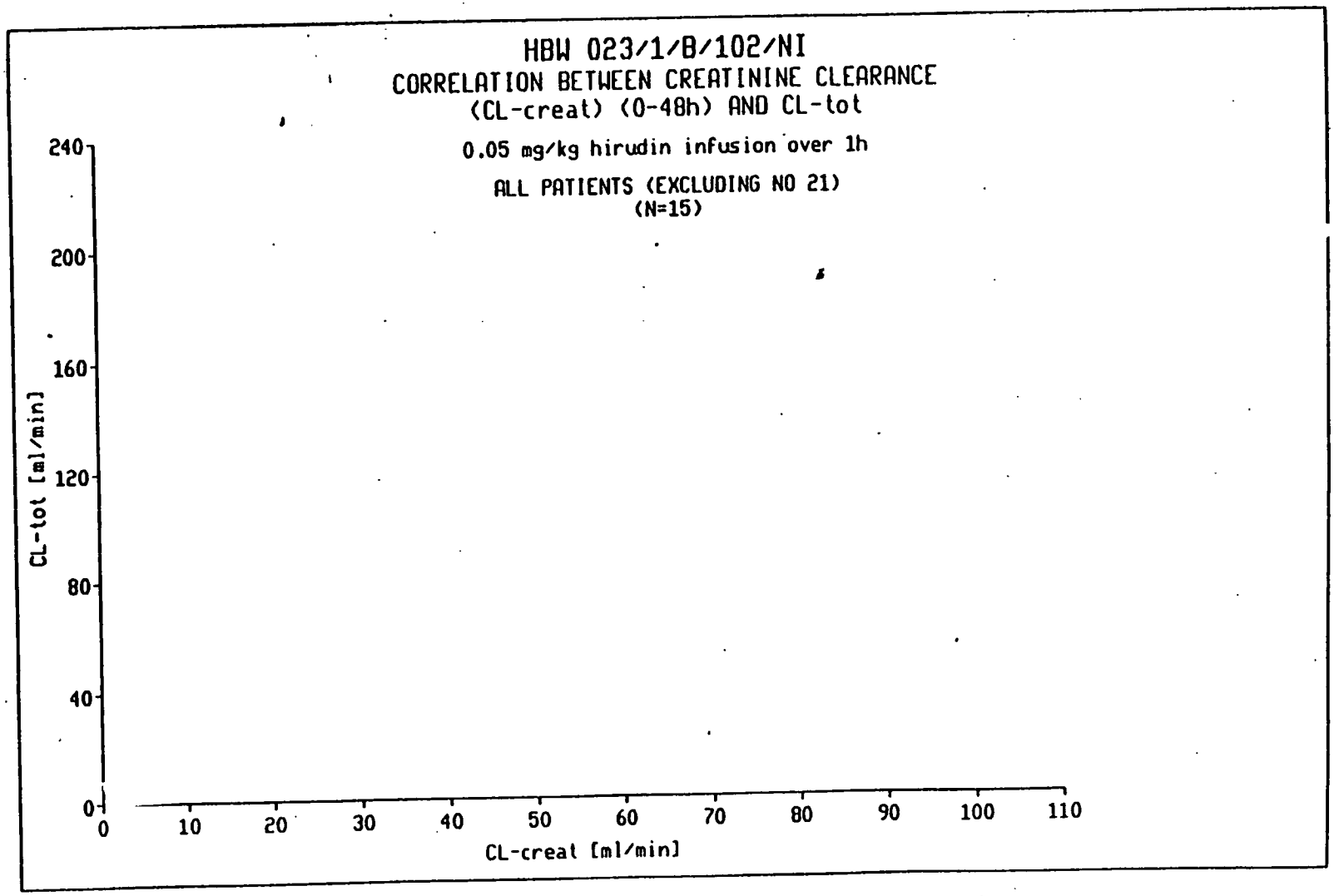

HBW 023/1/B/102/NI
CORRELATION BETWEEN CREATININE CLEARANCE
(CL-creat) (0-48h) AND CL-tot
0.05 mg/kg hirudin infusion over 1h
ALL PATIENTS (EXCLUDING NO 21)
(N=15)
CL-tot [ml/min]
240
200
160
120
80
40
0
0
10
20
30
40
50
60
70
80
90
100
110
CL-creat [ml/min]

Figure 8

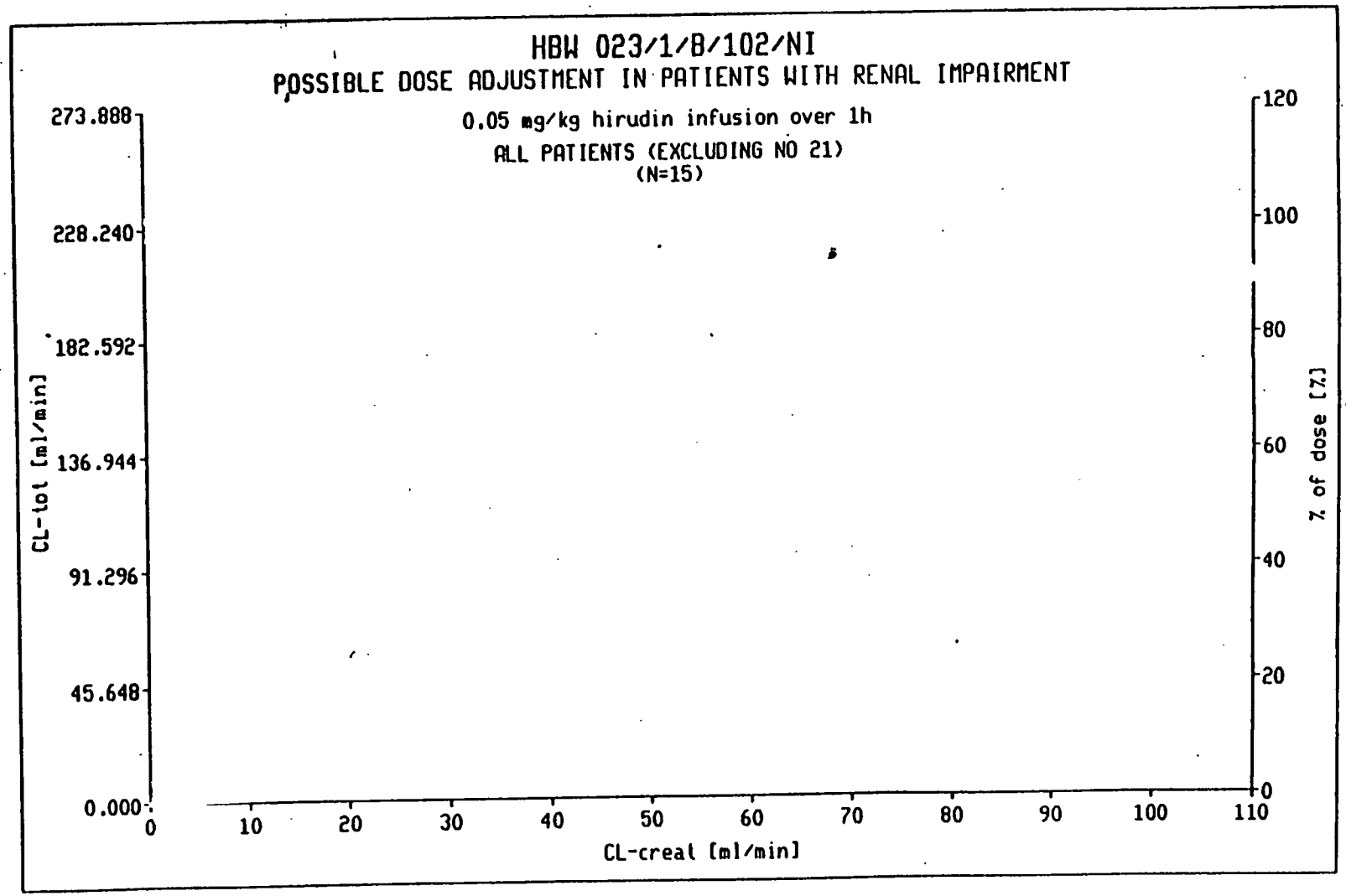

HBW 023/1/B/102/NI
POSSIBLE DOSE ADJUSTMENT IN PATIENTS WITH RENAL IMPAIRMENT
0.05 mg/kg hirudin infusion over 1h
ALL PATIENTS (EXCLUDING NO 21)
(N=15)
CL-tot [ml/min]
273.888
228.240
182.592
136.944
91.296
45.648
0.000
% of dose [%]
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
110
CL-creal [ml/min]

Figure 9

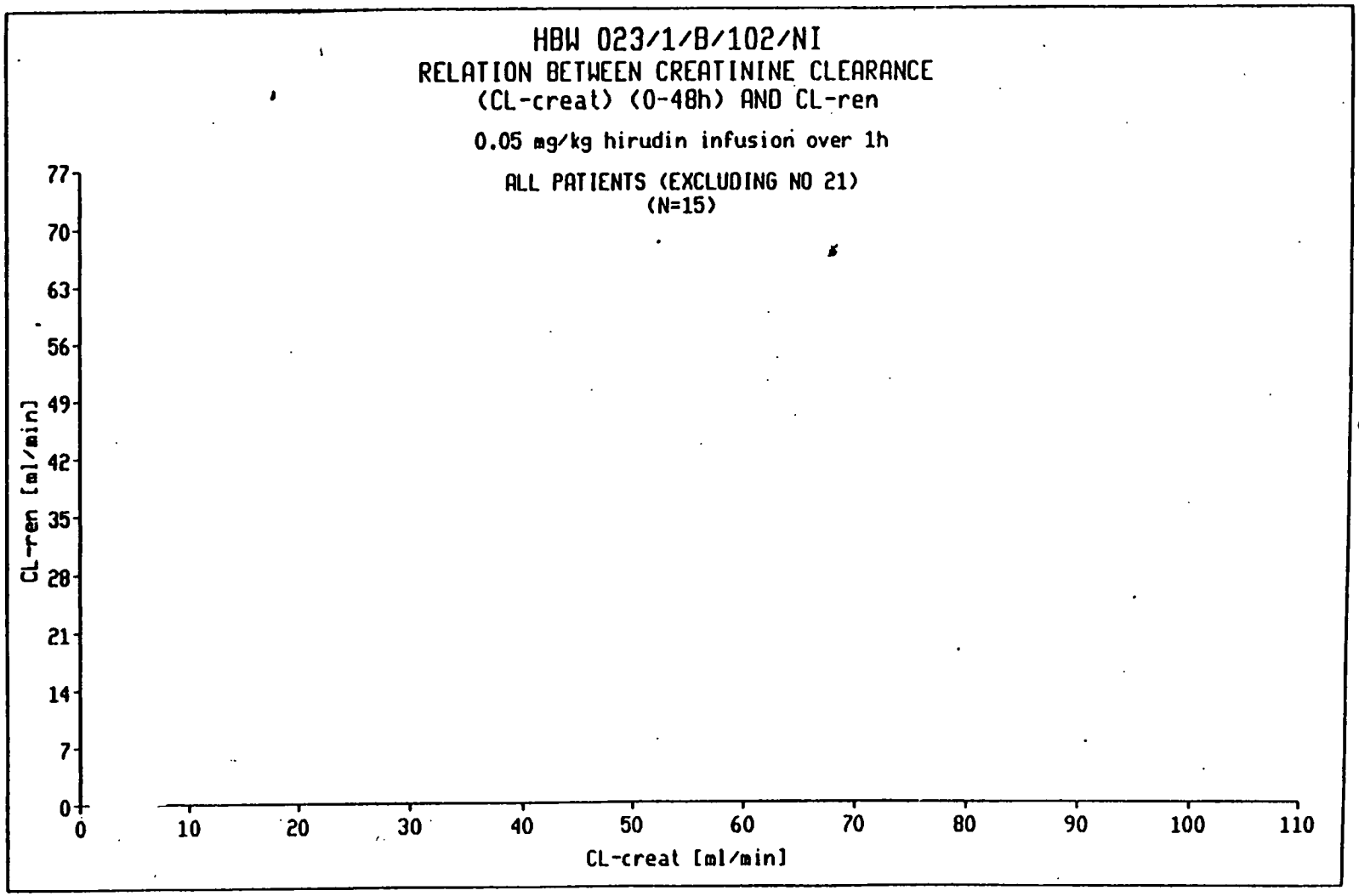

HBW 023/1/B/102/NI
RELATION BETWEEN CREATININE CLEARANCE
(CL-creat) (0-48h) AND CL-ren
0.05 mg/kg hirudin infusion over 1h
ALL PATIENTS (EXCLUDING NO 21)
(N=15)
CL-ren [ml/min]
77
70
63
56
49
42
35
28
21
14
7
0
0
10
20
30
40
50
60
70
80
90
100
110
CL-creat [ml/min]

Figure 10

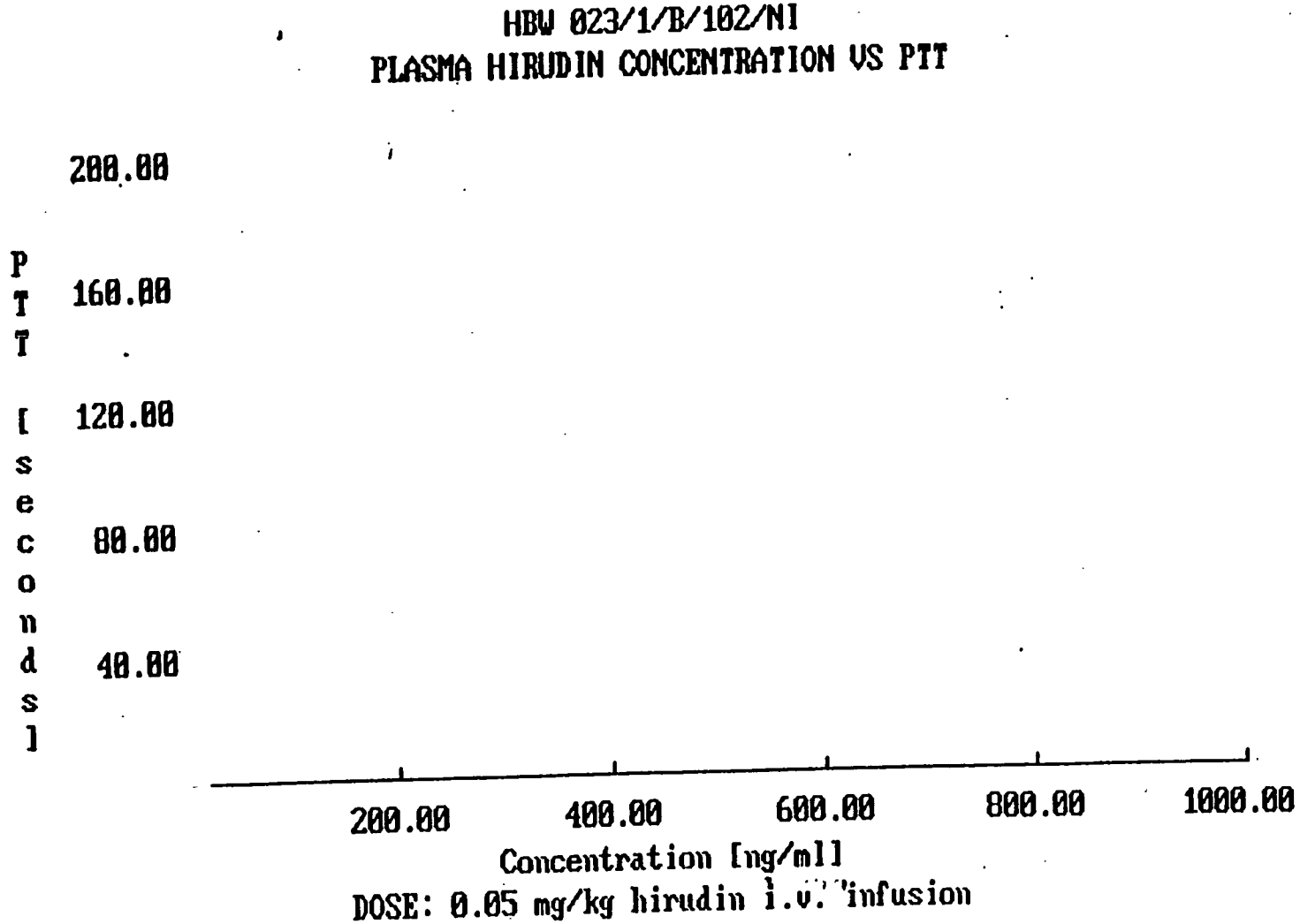

HBW 023/1/B/102/NI
PLASMA HIRUDIN CONCENTRATION VS PTT
200.00
160.00
120.00
80.00
40.00
PTT [seconds]
200.00
400.00
600.00
800.00
1000.00
Concentration [ng/ml]
DOSE: 0.05 mg/kg hirudin i.v. infusion

Figure 11

Population Pharmacokinetics of Lepirudin (r-Hirudin)

Studies included in this Analysis: A12, A4, A3, A14, B1, B2, B7, MN-201TH and MN-201-AP

Objective:
To characterize the population pharmacokinetics of r-hirudin and examine if there are significant demographic effects on its pharmacokinetic parameters.

Formulation: Hirudin lyophilisate for IV injection.

Study Design of Existing Data:

A12 This was a single dose pharmacokinetic study in which ten healthy male and female elderly subjects were to receive 0.1 mg/kg bolus injection of r-hirudin. Serial blood samples were to be collected up to 24 hours after medication for the determination of r-hirudin plasma concentration.

A4 This was a single dose pharmacokinetic study in which three groups of five healthy male subjects were to receive an IV infusion of 0.1, 0.15 or 0.2 mg/kg of r-hirudin over six hours. Serial blood samples were to be collected up to 24 hours after medication for the determination of r-hirudin plasma concentration.

A3 This was a single dose pharmacokinetic study in which 18 healthy subjects 9 males and 9 females) were to receive an IV bolus (2 minutes) of 0.1, 0.2 and 0.4 mg/kg of r-hirudin according to a randomized, three-way crossover design. Serial blood samples were to be collected up to 72 hours after medication for the determination of r-hirudin plasma concentration.

A14 This was a single dose pharmacokinetic study in which 16 subjects with varying degrees of renal impairment were to receive an one hour IV infusion of 0.05 mg/kg of r-hirudin. Serial blood samples were to be collected up to 120 hours after medication for the determination of r-hirudin plasma concentration.

B7 This was a clinical safety and efficacy study in which 82 patients 30 males and 52 females) with heparin-associated thrombocytopenia (HAT) were to receive initially various combination of r-hirudin IV boluses followed buy subsequent infusion Sparse blood samples were to be collected throughout the study for the determination of r-hirudin plasma concentrations.

MN-201TH

This was a clinical safety and efficacy study in which 155 patients 93 males, 62 females) with deep venous thrombosis (DVT) were to receive ascending

doses of r-hirudin subcutaneously every 12 hours up to five days (1st dose: 0.75 mg/kg, 2 nd dose: 1.25 mg/kg, 3 rd dose: 2.00 mg/kg). Sparse blood samples were to be collected throughout the study for the determination of r-hirudin plasma concentrations.

B1 This was a clinical safety and efficacy trial in which 143 patients 115 males, 28 females) suffering from acute myocardial infarction (AMI) were to receive an IV bolus of r-hirudin (0.1 - 0.4 mg/kg) followed by a constant rate infusion over 48 hours (0.06 to 0.15 mg/kg/hr). Serial blood samples were to be collected up to 72 hours after medication for the determination of r-hirudin plasma concentration.

B2 This was a clinical safety and efficacy trial in which 272 AMI patients 222 males, 50 females)received an IV bolus or r-hirudin followed by a constant rate infusion up to 72 hours). Serial blood samples were to be collected up to 84 hours after medication for the determination of r-hirudin plasma concentration.

APPEARS THIS WAY ON ORIGINAL

7MN-201-AP

This was a clinical safety and efficacy trial in which 61 patients with unstable angina (47 males , 14 females) were randomized to receive either a low dose (0.3 mg/kg bolus followed by sequential 24 hr constant infusions of 0.12 mg/kg/h and 0.04 mg/kg/h) or a high dose (0.5 mg/kg bolus followed by sequential 24 hour infusions of 0.24 mg/kg/hr and 0.04 mg/kg/hr) regimen of r-hirudin. Serial blood samples were to be collected up to 48 hours after medication for the determination of r-hirudin plasma concentration.

Assay:

Modeling Process:

Out of a total 773 patients, 644 produced 5,669 measurable r-hirudin concentrations from the nine studies. Thirty five of these concentrations were considered outliers and were not used in the modeling process. These outliers were either measurable concentrations before r-hirudin was actually administered or the concentrations were exceedingly high. Plasma r-hirudin concentration-time data were analyzed by nonlinear mixed-effects modeling (NONMEM, Version IV) to develop a population pharmacokinetic model using the first-order approximation method. The full model was built by identifying a base model and then adding significant covariates to the base model. A combination of exponential and/or additive error model was used to characterize the distribution of inter and residual variabilities.

A two-compartment model with rapid IV bolus injection or constant rate IV infusion was

Results:

Demographic profiles of the 644 healthy subjects and patients from the nine studies are summarized in the following table.

Patient/Subject Demographics Summary (N=644)	
Demography	Mean (SD)
Age (years)	56.83 (14.37)
Weight (kg)	77.58 (12.53)
Creatinine clearance (ml/min)	89.43 (33.86), N=634*
Gender (%)	70.3% male, 29.7% female
Race (%)	97.5% caucasians, 2.5% others
Aspirin concomitant treatment	60.4% yes, 39.6% no
tPA concomitant treatment	23.0% yes, 77.0% no
Streptokinase concomitant treatment	32.3% yes, 67.7% no

Coumarin concomitant treatment	12.0% yes, 88.0% no (Incorrect**)
Heparin concomitant treatment	16.8% yes, 83.2% no
*: There are 5 patients from study 7MN-201 with incorrect serum creatinine concentration units which led to creatinine clearance values of > 300 ml/min. These values are not included in this summary.	

Conclusion:

In conclusion, age, gender, creatinine clearance, total dose and PK drug-drug interactions were observed to influence the pharmacokinetics of r-hirudin. Specifically, following effects were observed:

Gender: CL in females is 25.5% lower than males. V1 values in female is 18.8% lower than male.

Age: CL is inversely related to age

Creatinine clearance: CL is proportionally related to creatinine clearance

Drug-Drug Interaction: ASA and heparin treated patients had V1 values of 116% and 277% higher than other patients, respectively. Streptokinase coadministration reduces V2 by 52.6%.

Comments:

The data set is composed of concentration values assayed by two different assays. Even though the sponsor studied the effect of assay on residual variability. A similar approach should have been used on CL parameter.

It is important to note that r-hirudin after administration does not stay as intact molecule. In fact it appears that this peptide molecule could be cleaved off of few terminal amino acids. This process could differ when drug is administered IV as oppose to SC.

In this population analysis the sponsor did not attempt any model validation. A good strategy would have been of randomly selected data. The model developed based on the rest of the data could then be tested for its prediction performance on the left-out data.

The sponsor claimed that 12% of 644 patients took r-hirudin together with coumarin. However this reviewer found only 2 patients out of 644 who took these two drugs together in this data base.

wt	id	anya	anyt	anvs	anvc	anyh
1st Qu.: 69.00	1st Qu.:201200	1:293	1: 59	1:175	1: 2	1: 38
Median : 78.00	Median :208400					
Mean : 77.58	Mean :372500					
3rd Qu.: 85.00	3rd Qu.:621000					

from mac "windin.data" file.

sex	race	age	wt	crcl	anvt
2:180	2: 4	1st Qu.:49.00	1st Qu.: 69.0	1st Qu.: 66.17	1: 59
	3: 50	Median :60.00	Median : 78.0	Median : 85.71	
	4: 4	Mean :57.43	Mean : 77.9	Mean : 89.01	
	5: 2	3rd Qu.:68.00	3rd Qu.: 85.0	3rd Qu.:105.60	

anya	anyn	anyc	anys	cl	vl
0:323	0:576	0:612	0:445		
1:291	1: 38	1: 2	1:169	1st Qu.: 5.573	1st Qu.: 6.971
				Median : 7.741	Median :12.240
				Mean : 7.755	Mean :11.560
				3rd Qu.: 9.594	3rd Qu.:15.070

v2	q
1st Qu.: 6.013	1st Qu.:6.098
Median :11.880	Median :6.098
Mean :10.740	Mean :6.098
3rd Qu.:12.980	3rd Qu.:6.098

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information

Pharmacokinetic Pharmacodynamic Evaluation of r-Hirudin

The effect of various lepirudin plasma concentrations an aPTT prolongation was studied in different patient populations including healthy volunteers (395 samples), thrombolyzed patients (1710 samples), HAT type II patients (580 samples) and other (1106 samples, mainly from DVT patients). Samples resulting in aPTT-ratios above 8.0 or below 0.5 were not taken into consideration.

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[1] Tripodi A., Chantarangkul V, Arbini A A, Moia M, Mannucci P M, Effect of hirudin on aPTT determined with ten different reagents. Thrombosis and Haemostasis 70, 286-288 (1993)

Appendix 1, Table 1 HBW 023 plasma concentration-effect relationship (details of regression analysis)

Population	Samples	$n_0 \pm SE$	$n_1 \pm SE$
Healthy volunteers	395	-2.328 ± 0.039	0.879 ± 0.014
Thrombolysis (rt-PA)	810	-0.824 ± 0.114	0.384 ± 0.038
Thrombolysis (SK)	900	-1.385 ± 0.101	0.586 ± 0.035
HAT type II	580	-1.918 ± 0.217	0.668 ± 0.069
Other	1106	-2.556 ± 0.141	0.824 ± 0.045

Appendix 1, Table 2 Model predicted aPTT-ratios for various populations

Plasma concentration (µg/ml)	Healthy volunteers	Thrombolysis		HAT type II	Other
		rt-PA	SK		
0.25	1.74	2.15	2.03	1.66	1.47
0.5	2.06	2.36	2.33	1.86	1.65
1.0	2.57	2.62	2.74	2.13	1.89
1.5	3.01	2.80	3.05	2.34	2.09
2.0	3.42	2.95	3.33	2.52	2.27

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Labeling